IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Beutler, et al.

Serial No.: 09/396,985

Filed: September 15, 1999

For: LPS-RESPONSE GENE COMPOSITIONS AND METHODS

Group Art Unit: 1646

Examiner: Basi, N.

Atty. Dkt. No.: UTSD:602

CERTIFICATE OF MAILING
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October 23, 2003
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Mark B. Wilson

APPEAL BRIEF

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Commissioner for Patents
PO Box 1450
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Commissioner:

Applicants hereby submit an original and two copies of this Appeal Brief to the Board of Patent Appeals and Interferences in response to the final Office Action dated December 18, 2002. By virtue of the Request for Extension of Time submitted herewith, this brief is due on October 23, 2003 (the Notice of Appeal having been received by the PTO on June 23, 2003). The $165.00 fee for filing this Appeal Brief is included herewith. If the check is inadvertently omitted, or should any additional fees under 37 C.F.R. §§ 1.16 to 1.21 be required for any reason relating to the enclosed material, or should an overpayment be included, the Commissioner is

authorized to deduct or credit the fees from or to Fulbright & Jaworski L.L.P. Deposit Account No.: 50-1212/UTSD:602/MBW.

I. REAL PARTY IN INTEREST

The real party in interest is the assignee, Board of Regents, The University of Texas System.

II. RELATED APPEALS AND INTERFERENCES

There are no appeals or interferences related to this case.

III. STATUS OF THE CLAIMS

Claims 38-40, 52-61, 63-68, 70-75, and 100-103 are currently pending. Appellants have submitted an amendment to claim 67 concurrently herewith. The amendment is made to resolve a minor editing issue, and no new matter is added. A copy of the claims as they will exist upon entry of the amendment to claim 67 is attached as Appendix 1 to this brief. A copy of the claims as they will exist if the amendment to claim 67 is not made is attached as Appendix 2 to this brief.

IV. STATUS OF AMENDMENTS

One set of amendments was submitted on February 13, 2003, following the final Office Action. The amendments were denied entry on the grounds that they were not deemed to place the application in better form for appeal by materially reducing or simplifying the issues for appeal.

An amendment to the claims is filed concurrent with this brief (copy attached as Exhibit A), which if entered amends claim 67 to resolve a minor editing issue. No new matter has been added.

V. SUMMARY OF THE INVENTION

The present invention is directed to methods of screening for modulators of a lipopolysaccharide-mediated response. Specification, p. 77, ln. 26 to p. 80, ln. 28.

VI. ISSUES ON APPEAL

Are claims 38-40, 52-61, 63-68, 70-75, and 100-103 definite under 35 U.S.C. § 112, second paragraph?

Are claims 38-40, 52-61, 63-68, 70-75, and 100-103 enabled under 35 U.S.C. § 112, first paragraph?

VII. GROUPING OF THE CLAIMS

The claims do not stand and fall together. With respect to the rejection of claims 38-40, 52-61, 63-68, 70-75, and 100-103 under 35 U.S.C. § 112, second paragraph, on the grounds that "TLR-4" is indefinite, Appellants submit that claims 39, 40, 57-61, 74, and 100 are separately patentable. Claims 39, 57-61, 74, and 100 each expressly recite the amino acid sequences of one or several homologous TLR-4 polypeptides or the nucleotide sequences encoding those polypeptides; and claim 40 is dependent on claim 39. These claims necessarily implicate the amino acid structure of TLR-4 polypeptides and therefore cannot be indefinite on the grounds asserted by the Action. Likewise, any other claim incorporating any such sequences could not be rejected on these grounds.

With respect to the rejection of claims 38-40, 52-61, 63-68, 70-75, and 100-103 for lack of enablement under 35 U.S.C. § 112, first paragraph, Appellants submit that claims 40, 55, and 56 are separately patentable. The Examiner has stated that the specification is enabled for a screening method for compounds which modulate a LPS mediated response by inducing the synthesis or altering expression of TLR-4 of SEQ ID NOS: 2, 4, 6, 98, and 99. Action, p. 6, ln. 18-20.

Claim 40 is directed to a screening method wherein a reporter gene is operatively positioned under the control of a nucleic acid segment comprising a promoter from a TLR-4 gene. In addition, claim 40 reads on a TLR-4 polypeptide that has the sequence of SEQ ID NOS: 2, 4, 6, 98, and 99. Appellants, therefore, submit that the enablement of claim 40 must be addressed separately.

Claims 55 and 56 are directed to screening methods wherein a putative modulator affects the transcription of TLR-4 and the translation of TLR-4, respectively. Appellants, therefore, submit that the enablement of claims 55 and 56 must be addressed separately.

VIII. ARGUMENT

A. Substantial Evidence is Required to Uphold the Examiner's Position.

Findings of fact and conclusions of law by the U.S. Patent and Trademark Office must be made in accordance with the Administrative Procedure Act, 5 U.S.C. § 706(A), (E), 1994. *Dickinson v. Zurko*, 527 U.S. 150, 158 (1999). Moreover, the Federal Circuit has held that findings of fact by the Board of Patent Appeals and Interferences must be supported by "substantial evidence" within the record. *In re Gartside*, 203 F.3d 1305, 1315 (Fed. Cir. 2000). In *In re Gartside*, the Federal Circuit stated that "the 'substantial evidence' standard asks whether a reasonable fact finder could have arrived at the agency's decision." *Id.* at 1312.

Accordingly, it necessarily follows that an Examiner's position on Appeal must be supported by "substantial evidence" within the record in order to be upheld by the Board of Patent Appeals and Interferences.

B. The Claims are Definite Under 35 U.S.C. § 112, Second Paragraph.

Claims 38-40, 52-61, 63-68, 70-75, and 100-103 are rejected under 35 U.S.C. § 112, second paragraph, as indefinite. Three grounds are asserted for this rejection: (i) that the term TLR-4 is insufficiently defining of the TLR-4 polypeptides of the invention; (ii) that the phrase "lipopolysaccharide mediated response" is unclear; and (iii) that the phrase "small molecule inhibitor" is indefinite. Appellants respectfully traverse all grounds for rejection.

1. The Legal Standard under 35 U.S.C. § 112.

A proper evaluation of the claims under the second paragraph of 35 U.S.C. § 112 requires that the claims be read in light of the specification as interpreted by one of ordinary skill in the art. *North Am. Vaccine, Inc. v. American Cyanamid Co.*, 7 F.3d 1571, 1579, 28 USPQ2d 1333, 1339 (Fed. Cir. 1993); *In re Moore*, 439 F.2d 1232, 1235 (C.C.P.A. 1971). Furthermore, the law does not require that only immutable or invariant terms be used in claim language. Inventors are encouraged to use concise language, as long as it is reasonably definite in view of the specification. This is long established law. *North Am. Vaccine, Inc.*, 7 F.3d 1571 at 1579; *Miles Lab., Inc. v. Shandon, Inc.*, 997 F.2d 870, 875, 27 USPQ2d 1123, 1126 (Fed. Cir. 1993); *Loom Co. v. Higgins*, 105 U.S. (Otto.) 580, 586 (1881).

2. Appellants have defined the term TLR-4 in light of the specification and the skill of the ordinary artisan.

a) The claims are clear and definite in light of the specification.

Appellants have provided a detailed and consistent definition of TLR-4 in the specification. Most particularly, TLR-4 as used by the Appellants in describing particular

embodiments refers explicitly to polypeptides of the sequences of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:98 or SEQ ID NO:99 and those sequences at least about 85% similar thereto or biologically functional equivalents thereof. Specification, p. 30, ln. 4-15; p. 73, ln. 18 to p. 76, ln. 11; and Example 8, p. 105-122.

In addition, the specification discloses structural and functional properties of TLR-4. For example, Table V on page 119 of the specification illustrates the percent homology among six species (human, chimpanzee, baboon, mouse, hamster, and rat) at four functional domains (extracellular, transmembrane, proximal cytoplasmic, and distal cytoplasmic) of TLR-4. This demonstrates that the family of TLR-4 receptors share high sequence similarities in specific domains. The specification also provides abundant support showing that TLR-4 is essential for LPS signaling. *See* Example 7, p. 100-05; Example 9, p. 123-30. Methods by which a TLR-4 mediated response to LPS can be assayed are also provided by the specification. Example 1, p. 87-88; Example 2, p. 95-96.

In view of the properties and structures of TLR-4 polypeptides supplied in the specification, Appellants submit that the specification sheds sufficient light upon the present claims to render them clear and definite to one of skill in the art under the second paragraph of 35 U.S.C. § 112.

b) The term TLR-4 is well known to those skilled in the art.

Appellants also point out that the law does not require that the Appellants define in the specification every term of art well known to the artisan. Use of a well known term of art in the specification without detailed definitions thereof does not render claims utilizing that same language indefinite. *W.L. Gore & Assoc., Inc. v. Garlock, Inc.*, 721 F.2d 1540, 1556-58, 220 USPQ 303, 315-16 (Fed. Cir. 1983). Claims may, therefore, make use of the language understood by those of skill in the art without additional, detailed definitions in the written

description. *Id.* Where later developed terms are arguably more definite than the terms available near the filing date, later accepted and more precise terms are favored. *In re Fisher*, 427 F.2d 833, 838 (C.C.P.A. 1970). The name TLR-4 is in common use among artisans in the field in exactly the sense in which the Appellants have defined it. Although the referenced polypeptide may have once been named Toll-4, use of the more current and precise term TLR-4 (or TLR4) cannot render the claims indefinite. *See Id.*

Indeed, the identity of TLR-4 within the family of Toll-like receptors is unambiguous, as is known to those of skill in the art. Appellants have provided the Examiner with numerous examples of the knowledge and skill in the art with respect to the definition of TLR-4 polypeptides. These examples include scientific literature displaying the knowledge available to one of skill in the art to define and recognize TLR-4 polypeptides. A list of references in the art using TLR-4 to refer to the polypeptides as the Appellants have defined them in the specification is attached as Exhibit B.

The Examiner was also provided with the declaration of Dr. David D. Chaplin (Exhibit C), which states that the specification as filed provides sufficient structural and functional properties by which to identify a protein as TLR-4 or its homolog.

Appellants point out that these facts should not be lightly dismissed absent any showing of a factual or scientific basis to doubt their veracity. Yet the Examiner continues to hold the position that "[t]he art nor specification disclose the structural and functional properties which must be present for the polypeptide to be classified as a TLR-4 polypeptide." Action, p. 3, ln. 12-13. However, the Examiner has not provided any evidence to refute the abundance of evidence submitted by Appellants. Absent a factual or scientific basis to the contrary, which the

Examiner has not provided, the term "TLR-4" must be found to be sufficiently defining of the TLR-4 polypeptides of the invention.

c) Conclusion

Appellants submit, that in view of the above, one of ordinary skill in the art would find the language of the claims definite under 35 U.S.C. § 112, second paragraph. Appellants, therefore, request that the Board overturn the rejection.

3. Claims 39, 40, 57-61, 74, and 100 are separately patentable.

As discussed above, the Action rejects claims 38-40, 52-61, 63-68, 70-75 and 100-103 on the grounds that "the name TLR-4 has not been defined in the claims and specification so as to allow the metes and bounds of the claims to be determined." The Action at p. 2, ln. 10-11.

Appellants submit that the asserted grounds for rejection, cannot apply to claims 39, 40, 57-61, 74 and 100. Claims 39, 57-61, 74 and 100 expressly recite the amino acid sequences of several homologous TLR-4 polypeptides or the nucleotide sequences encoding those polypeptides, and claim 40 is dependent on claim 39. These claims necessarily implicate the amino acid structure of TLR-4 polypeptides and therefore cannot be indefinite on the grounds asserted by the Action, even if the term "TLR-4" were indefinite, which it is not. Appellants, therefore, request that the Board overturn the rejection.

4. The term "Lipopolysaccharide mediated response" is clear, definite, and well known to those of skill in the art.

Claims 38, 40, 52 and 101-103 are rejected on the grounds that they are indefinite because the phrase "lipopolysaccharide mediated response" is allegedly not clear. Appellants respectfully traverse.

a) Lipopolysaccharide mediated responses have been well known in the art for decades.

The Action asks "where does the lipopolysaccharide pathway begin and end?" The Action, p. 3, ln. 11-12. Appellants point out that, as is well known in the art, responses to the presence of endotoxin (LPS) occur at the subcellular, cellular, tissue and organismal levels. Appellants draw attention to the specification beginning at line 19 of page 2 and extending through line 5 of page 3 (emphasis added):

> An important case in point concerns Gram-negative bacteria, all of which bear endotoxin (lipopolysaccharide, LPS) molecules in their outer membrane, which trigger a strong immune response on the part of the host which produces a shock-like syndrome, characterized by low blood pressure and hyporeactivity to vasoconstricter agents.
>
> When macrophages are exposed to pure preparations of endotoxin, they secrete numerous cytokine mediators, including tumor necrosis factor (TNF, TNFα), interleukin-1 (IL-1), interferon-α/β, GM/CSF, IL-8 and ultimately smaller "autocoid" molecules, all of which mediate an intense inflammatory reaction. Endotoxin recognition acts as an early warning signal through which a host may mount a timely defense against invasion by Gram-negative organisms. However, widespread activation of macrophages by endotoxin results in the development of septic shock. By most estimates, Gram-negative septic shock is responsible for 100,000 deaths per year in the United States alone. The entire syndrome of hypotension, coagulopathy, pulmonary edema and acute renal failure results, in large part, from the release TNF and other cytokines in response to exposure to endotoxin.

It is possible to measure such responses at the organismal level in experimental organisms. For example:

> Thirty years ago, mice of the C3H/HeJ strain were noted to be specifically and globally unresponsive to endotoxin, while closely related animals of the C3H/HeN or C3H/OuJ substrains exhibited normal responses (Sultzer, 1968). The median lethal dose of endotoxin is more than 100-fold higher in C3H/HeJ mice than in either of these other strains. Macrophages of C3H/HeJ mice fail to produce cytokines in response to endotoxin, and B-lymphocytes of C3H/HeJ mice are not driven to proliferate by endotoxin. While C3H/HeJ mice are highly resistant to the lethal effect of endotoxin, they are unusually sensitive to infection by gram-negative organisms. The mean lethal inoculum with *Salmonella typhimurium*, for example, is two organisms in C3H/HeJ mice, whereas several thousand organisms are required to kill mice of the C3H/HeN strain.

Specification, p. 4, ln. 4-16 (emphasis added). As Appellants have well documented in the specification, they have disclosed that the single gene that underlies resistance to exposure to endotoxin in the mouse strain C3H/HeJ encodes TLR-4 and that TLR-4 is ***the*** LPS receptor. TLR-4 is indispensable to the signaling that results in the LPS mediated responses. Thus, modulation of native TLR-4 activity in the cellular response to LPS necessarily modulates the LPS response. Therefore, through the application of a putative modulator of TLR-4 signaling (as provided by the Appellants), one may determine if the particular LPS response has, in fact, been modulated in the above example by observing whether the mouse lives or dies. In this particular, albeit extreme embodiment, Appellants are at a loss to know how it can be uncertain or unclear just exactly what responses may be expected to be manifest after exposure to LPS.

b) The Action mischaracterizes statements made by Dr. Chaplin in asserting grounds for rejection.

In response to the assertions in the Action (page 5, lines 5 and 6) that Appellants have disclosed "some of the 'actors and elements of lipopolysaccharide mediated response,'" (emphasis added) Appellants point out that the declaration of Dr. Chaplin has been improperly quoted. The proper quote reads "The specification clearly sets forth the actors and elements of lipopolysaccharide mediated responses that are mediated by TLR-4." Declaration of Dr. Chaplin, paragraph 5 (emphasis added).

No ambiguity exists in the statement of the declaration. Rather, the ambiguity has been introduced by the Examiner, who replaces Dr. Chaplin's "the actors" with the Action's "some actors." Since there is no basis provided for so modifying Dr. Chaplin's statement, any rejection based upon this misstatement of fact must be in error.

Furthermore, the Examiner has not provided any factual or scientific basis to refute the facts presented in the declaration. Instead, the Examiner merely quoted from the declaration and

then dismissed it without providing any basis for finding it not persuasive. Action, p. 4, ln. 19 to p. 5, ln. 7. Appellants point out that the declaration of Dr. Chaplin should not be lightly dismissed absent any showing of a factual or scientific basis to doubt its veracity.

c) The Action mischaracterizes Appellants' disclosure in asserting grounds for rejection.

The Action quotes the specification out of context in order to impugn the clarity and importance of Appellants' discovery and the claimed invention. The Action cites the last paragraph of page 3, but only quotes a single sentence. For convenience and accuracy, Appellants herein reproduce the entirety of the cited paragraph (quoted passage in italics):

> Endotoxin is known to trigger both tyrosine and serine phosphorylation events within the macrophage cell, and at least in part, *ras*, *raf*, *MEK*, and members of the MAP kinase family are also involved in signal transduction (Geppert *et al.*, 1994). The endpoints of endotoxin signaling include activation of the transcription of TNF and various genes, and activation of the translation of TNF mRNA (Beutler *et al.*, 1986; Han *et al.*, 1990). At the protein level, this stimulation by endotoxin leads to a several thousand-fold augmentation of cytokine biosynthesis by a macrophage cell. But the initial controlling element and event in the signaling pathway of macrophage response to endotoxin has not been identified. *Thus, in spite of its importance, most of the endotoxin signaling pathway remains relatively unknown.* Recently however, the Toll-like receptor 2 (TLR2) has been suggested to partially mediate lipopolysaccharide-induced cellular signaling (Gerard, 1998; Yang *et al.*, 1998).

In the context of the full paragraph it is clear that the quoted statement refers to the impediment to research imposed by the lack of identification of the "initial controlling element" in the pathway of macrophage response to endotoxin (LPS). Indeed, this paragraph occurs in the description of related art and describes the state of the art ***prior*** to the disclosure by the Appellants of the *Lps* gene, *i.e.* TLR-4. Moreover, it is the signaling pathway, at which TLR-4 is at the core, that was unknown, not LPS mediated responses, which have been known to those in the art for decades (see above). And further, Appellants' specification discloses the key actor at the core of LPS mediated responses, TLR-4.

d) Mischaracterizations by the Examiner do not render the claims indefinite.

The mischaracterization of the state of the art and the statements made by Dr. Chaplin cited above cannot rise to the task of demonstrating that the present claims are indefinite for reciting a "lipopolysaccharide mediated response." Indeed, given the mature state of the art relevant to lipopolysaccharide mediated responses, the ordinary artisan would immediately understand the nature and scope of the claimed methods.

e) The Term "Lipopolysaccharide mediated response" has an established meaning in the art.

Appellants emphatically reiterate that the law does not require that the Appellants define in the specification every term of art well known to the artisan. Use of a well known term of art in the specification without detailed definitions thereof does not render claims utilizing that same language indefinite. *W.L. Gore & Assoc., Inc.* 721 F.2d at 1556-58. If necessary, a standard reference work may inform the reading of the specification, and if so, that in itself does not render claims utilizing that language indefinite. *Atmel Corp. v. Information Storage Devices, Inc.*, 198 F.3d 1374, 1382 (Fed. Cir. 1999) ("even a dictionary or other documentary source may be resorted to...").

Indeed, the content of such a standard reference work is intrinsic evidence of the meaning of claim terms. The Court of Appeals for the Federal Circuit has said:

> Dictionaries, encyclopedias and treatises, publicly available at the time the patent is issued, are objective resources that serve as reliable sources of information on the established meanings that would have been attributed to the terms of the claims by those of skill in the art. Such references are unbiased reflections of common understanding not influenced by expert testimony or events subsequent to the fixing of the intrinsic record by the grant of the patent, not colored by the motives of the parties, and not inspired by litigation. Indeed, these materials may be the most meaningful sources of information to aid judges in better understanding both the technology and the terminology used by those skilled in the art to describe the technology.

Texas Digital Sys., Inc. v. Telegenix Inc., 308 F.3d 1193, 1202-3 (Fed. Cir. 2002).

Appellants have previously supplied the Examiner with passages from MEDICAL MICROBIOLOGY 130-33 (S. Baron ed.) (4th ed. 1996) (Exhibit D). This work is an example of a standard reference or treatise that verifies that a lipopolysaccharide mediated response is well understood by those of skill in the art. The passages are a summary of the extensive literature in the field of endotoxin biology as it existed in 1996, and they demonstrate that one of skill in the art would know the metes and bounds of a LPS mediated response. As indicated in the Appellants' specification at page 2, line 20, and as is well known and readily apparent to one of skill in the art, lipopolysaccharide is a term synonymous with endotoxin. *See* MEDICAL MICROBIOLOGY at 130, col. 2, par. 4.

Further, and as is well known and readily apparent to one of skill in the art, "The biologic effects of endotoxin [LPS] have been extensively studied." MEDICAL MICROBIOLOGY at 131, col. 1, under heading "Biologic Activity of Endotoxin." Thus, LPS-mediated responses at least include (as of 1996) those activities listed in Table 7-4, page 132 of MEDICAL MICROBIOLOGY. Indeed, the central importance of LPS in the mediation of cellular responses to a variety of conditions is clear and well known to the ordinary artisan.

At the time of this summary (1996), the specific mechanism of cellular response was not clear. *See* MEDICAL MICROBIOLOGY at 132, col. 1-2. What was clear was that the host cell exposure to endotoxin was key to the "myriad sequelae" that exposure to endotoxin produces. "It does seem clear that the host cellular response to endotoxin, rather than a direct toxic effect of endotoxin, plays the major role in causing tissue damage." MEDICAL MICROBIOLOGY at 132, col. 2. Indeed, it is the Appellants' discovery that TLR-4 polypeptide plays the key role as a cell's LPS receptor and is, therefore, the key in the pathway leading to the various responses to certain

types of infection. "[T]he demonstration that *Lps* is identical to TLR-4 leaves no room for doubt that TLR-4 is essential for LPS signaling." Specification, p. 104, ln. 14-15. This discovery, therefore, identifies ***the*** pathway, *i.e.* through TLR-4, by which these responses are mounted.

f) The term "small molecule" is definite.

The Action rejects claims 101-103 as indefinite under 35 U.S.C. § 112, second paragraph, on the grounds that the term "small molecule inhibitor" is unclear. Appellants traverse this rejection. The term "small molecule inhibitor" has a well established meaning in the art. The law does not require that the Appellants define in the specification every term of art well known to the artisan. Use of a well known term of art in the specification without detailed definitions thereof does not render claims utilizing that same language indefinite. *W.L. Gore & Assoc., Inc.* 721 F.2d at 1556-58.

A skilled researcher would understand that, in the context of the instant specification and claims, the term "small molecule inhibitor" describes low molecular weight, non-peptide inhibitors. In support of Appellants' argument, Appellants provide the second Declaration of Dr. David D. Chaplin (Exhibit E), a skilled artisan in the field of endotoxins and cellular biology. Dr. Chaplin states that the term "small molecule inhibitor" describes low molecular weight, non-peptide inhibitors, and that this definition is accepted in the art. The second Declaration of Dr. Chaplin also provides the results of a search in PubMed, a database of relevant scientific literature, that revealed 61 references in which the words "small molecule" and "inhibitor" appeared in the title alone. This evidences that skilled researchers understand the meaning of the term "small molecule inhibitor."

Appellants, therefore, submit that the term "small molecule inhibitor" is definite as it is used in the specification and claims. Appellants respectfully request that the Board overturn the rejection.

5. Summary

Appellants submit that the claims are definite under the second paragraph of 35 U.S.C. § 112 when properly viewed in light of the ordinary skill of one in the relevant art and the detailed descriptions available to the artisan in the Appellants' specification. Appellants, therefore, respectfully request that the Board overturn the rejections.

C. The Claims are Enabled Under 35 U.S.C. § 112, first paragraph.

The Action rejects claims 38-40, 52-61, 63-68, 70-75 and 100-103 under the first paragraph of 35 U.S.C. § 112. The Examiner has stated that the specification is enabled for a screening method for compounds which modulate a LPS mediated response by inducing the synthesis or altering expression of TLR-4 of SEQ ID NOS: 2, 4, 6, 98, and 99. Action, p. 6, ln. 18-20. However, the Examiner argues that other methods of screening for modulators of LPS-mediated responses through their interaction with TLR-4 are not enabled. Appellants respectfully submit that all of the claimed methods are enabled.

1. The Legal Standard of Enablement

To be enabling within the meaning of 35 U.S.C. § 112, the application must contain a description sufficient to enable one skilled in the art to make and use the claimed invention without unduly extensive experimentation. *Atlas Powder Co. v. E.I. du Pont De Nemours & Co.*, 750 F.2d 1569, 1576, 224 USPQ 409, 413 (Fed. Cir. 1984). Furthermore, it is well settled that the Examiner has the initial burden of producing reasons that substantiate a rejection based on lack of enablement. *See In re Marzocchi,* 439 F.2d 220, 224, 169 USPQ 367, 370 (C.C.P.A. 1971); *In re Wright,* 999 F.2d 1557, 1561, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993). The Examiner's burden requires that the Examiner supply a factual basis or scientific principle to

reasonably doubt the accuracy of a clear disclosure *In re Marzocchi*, 439 F.2d 220, 224, 169 USPQ 367, 370 (C.C.P.A. 1971).

2. The Action supplies no factual or scientific principle to reasonably doubt Appellants' disclosure.

Appellants contend that the Examiner has not carried the burden of establishing a *prima facie* case of lack of enablement. To the contrary, the Examiner has demanded unnecessary claim limitations and posited a number of factually unsupportable allegations regarding the nature and function of the claimed methods and TLR-4 in particular.

3. The Action misconstrues the invention in asserting lack of enablement.

The Action demands that the method disclose "how the modulator targets a TLR-4 polypeptide and if the response being measured is in fact a lipopolysaccharide mediated response, since there is [sic] no controls to compare the results to." The Action at p. 7, ln. 14-16.

Appellants point out that the pending claims are directed to methods of ***screening*** for modulators of a lipopolysaccharide mediated response that compare the response before and after contact of TLR-4 with a ***putative*** modulator or ***candidate*** substance. As such, the method does not require that the exact mode of action of any successful modulator so identified be determined ***prior*** to the screening procedure, as the Action demands. Indeed, if the artisan knew the identity of the modulator and its mode of action, there would be no need to practice the present invention. The present invention is directed to methods of identifying such modulators, whose identity would, of course, be unknown absent the present invention.

Furthermore, as presented above, the nature and scope of a lipopolysaccharide-mediated response is clear to the artisan. The assertions that there "is no controls [sic]" ignores the limitations of the claims. The claims specify the comparison between the measured LPS response ***prior*** to the contact with TLR-4 by the modulator with the measured LPS response

after contact with TLR-4 by the putative modulator, wherein a difference in the lipopolysaccharide mediated responses indicates that the putative modulator is a modulator of a lipopolysaccharide mediated response. *See* claim 38.

In addition, the Examiner's assertion that "cell death may be mediated by many different signaling pathways, it is not necessarily a lipopolysaccharide mediated response" is illogical in the context of the claimed invention. Action, p. 7, ln. 19-20. The response being measured is the result of a cell's exposure to lipopolysaccharide. This necessarily means that the response is lipopolysaccharide mediated. The Examiner has provided no factual basis to support the assertion that a cell's response to the presence of lipopolysaccharide would be mediated by a signaling pathway other than the lipopolysaccharide signaling pathway.

4. Unsupported, or unsupportable allegations do not rise to the level of factual evidence or scientific principle.

Additionally, the allegation that the LPS-mediated response mediated by TLR-4 could be due to some other pathway (the Action at p. 7, ln. 18) simply ignores the nature of Appellants' discoveries and the extensive disclosure provided regarding the central, indispensable role of TLR-4 polypeptides in LPS-mediated responses. The Action cites no reference, nor supplies any Examiner's affidavit. Indeed, the Action supplies no factual basis at all for doubting the veracity of Appellants' discovery and disclosure. In fact, the general acceptance and importance of Appellants' discovery to those in the art is reflected in the extensive literature already made of record in the present application, and which refers to and builds upon Appellants' work. The central role of TLR-4 in the signaling pathway is now widely accepted by the field. Appellants have made of record publications acknowledging Appellants' discovery and the role of TLR-4 in LPS responses. For example, see Bochkov *et al.*, *Protective Role of Phospholipid Oxidation Products in Endotoxin-Induced Tissue Damage*, NATURE 419:77-81 (2002) (Exhibit F).

5. *Appellants supply abundant factual evidence of enablement.*

Appellants point to the specification at page 2, line 15 through page 4, line 25, and page 22 lines 3-8 for a succinct description of the events and circumstances that comprise the induction of a response to LPS and the resultant effects that may be measured as indicative of an LPS-mediated response. Further, exemplary parameters and methods for measuring and determining the LPS-mediated responses are found in several locations in the specification. Specifically, splenocyte proliferation assays are described in Example 1, p. 87, ln. 8-24, and in Example 2, p. 95, ln. 25 to p. 96, ln. 17. Assays for TNF production in peritoneal macrophages are described in Example 1, p. 87, ln. 26 to p. 88, ln. 15, and in Example 2, p. 95, ln. 25 to p. 96, ln. 17. Assays for TNF production in murine macrophages expressing normal TLR-4 and various mutant forms of TLR-4 are described in Example 9, p. 123-29.

All of these responses may be utilized in the practice of the presently claimed methods by those of skill in the art, and the choice of which to use is a matter of preference and circumstance. Furthermore, all of these responses are well recognized by those of skill in the art as a "lipopolysaccharide mediated response" as evidenced by the numerous factual materials, including the declaration of Dr. Chaplin, made of record in this application.

As mentioned above, the specification provides specific examples of how to measure the LPS-mediated response mediated by TLR-4. In the section of the Specification titled "Assays for LPS responsiveness" two common examples of LPS-mediated response assays are described: a splenocyte proliferation assay and a macrophage response assay. *See* Example 1, p. 87-88. The splenocyte response assay compares the proliferation of splenocytes incorporating tritiated thymidine (as measured by counts per minute, CPM) with and without stimulation with LPS. The macrophage response assay measures the percent of cytotoxicity due to TNF released by cells in response to LPS.

These are exemplary parameters and methods for measuring the LPS response. Further, and in direct contradiction to the assertions made by the Examiner, claims 63 and 64 expressly recite TNF secretion as one LPS inducible response that may be measured. Therefore, the specification and the claims as filed provide concrete examples, methods, and standards for measuring responses induced by LPS and mediated through TLR-4. See paragraph 15 of the declaration of Dr. Chaplin, which provides verification of this fact by one of skill in the art.

Appellants have repeatedly called the Examiner's attention to these passages in the specification, which describe and demonstrate specific LPS-mediated responses that may be measured in the implementation of the present invention and are well known to the artisan. Yet the Examiner has failed to present any fact or scientific theory to refute this evidence. In the absence of any factual basis or scientific principle sufficient to doubt the substantial body of factual and scientific evidence demonstrating that the ordinary artisan may make and use the claimed invention without undue experimentation, Appellants submit that the present claims are enabled under 35 U.S.C. §112.

6. The Action misconstrues the invention and Appellants' arguments.

The Action maintains that there is but a single embodiment of the present invention that is enabled: a screening method for compounds that modulate a LPS-mediated response by inducing the synthesis or altering the expression of TLR-4 of SEQ ID NOS: 2, 4, 6, 98, or 99. Action at p. 6, ln. 18-20. Although altered expression of TLR-4 of SEQ ID NOS: 2, 4, 6, 98, or 99 (or a reporter gene, see claim 40) may be ***one*** mode of LPS response that is measured, it is not the sole means of measuring TLR-4 mediated LPS responses disclosed by the specification. As described above, the specification also discloses two other examples of LPS-mediated response assays: a splenocyte proliferation assay and a macrophage response assay.

With regard to this issue, Appellants note that the Examiner continues to misconstrue Appellants' arguments submitted in previous responses. In particular, the Action states "Applicant argues LPS response disclosed by the specification is by far not the sole means of modulating the TLR-4 response." The Action at page 7, lines 10-12. This statement confuses the LPS-mediated response and the role of putative or identified modulators of TLR-4's role in the response. The statement appears to equate LPS responses with modulators of TLR-4. As is readily apparent to those of skill in the art, however, this assertion is simply incorrect in view of the specification and the relevant literature.

The specification and the arguments presented by Appellants in previous responses make clear that TLR-4 is essential in the signaling pathway that results in, for example, an increase in TNF production as a result of lipopolysaccharide exposure to cell surface receptors and other well known LPS responses, including death. The LPS response is, therefore, largely "downstream" of the action of TLR-4 polypeptides at the cell surface. Modulation of LPS response through the modulation of TLR-4 activity, not merely its expression, is described and enabled by the specification as understood by those of skill in the art.

The Action nevertheless demands that "[f]or the person of ordinary skill in the art to screen for modulators of LPS mediated response by any other means that those disclosed as 'enabling' above [referring to assaying TLR-4 synthesis or expression], the artisan must first isolate other proteins capable of direct or indirect interaction with LPS and its modulators, and develop screening assays to determine if certain compounds can be modulators of the LPS mediated response." The Action at p. 8, ln. 13-17.

Appellants are simply at a loss to understand where the requirement for isolation of "other proteins capable of direct or indirect interaction with LPS and its modulators" is to be

found in the art or the disclosure of the specification. If the Action intends to refer to previous arguments of the Appellants, which noted that the sensitivity of the LPS-mediated response may be directly effected by the endogenous levels of TLR-4 expression, but that the LPS response itself may also be modulated by the nature of the physical interactions of TLR-4 with the other components of the pathway, Appellants respectfully point out that the mention of known modulators of TLR-4 sensitivity was provided to illustrate that the action of TLR-4 in initiating LPS-mediated responses was not dependent upon the up-regulation or down-regulation of its expression.

Modulation of LPS response may occur through the modulation of TLR-4 activity, not merely TLR-4 expression levels. This core concept demonstrates the enablement of the present invention, which provides that further, additional modulators may be identified using the claimed methods of screening. With regards to any need to "develop screening assays to determine if certain compounds can be modulators of the LPS mediated response" Appellants respectfully note that this is exactly the problem solved by the presently claimed methods. Again, there is no need to *a priori* identify modulators in order to practice a method that acts to identify such modulators.

7. Claims 40, 55, and 56 are separately patentable.

With respect to the rejection of claims 38-40, 52-61, 63-68, 70-75, and 100-103 for lack of enablement under 35 U.S.C. § 112, first paragraph, Appellants submit that claims 40, 55, and 56 are separately patentable. The Examiner has stated that the specification is enabled for a screening method for compounds which modulate a LPS mediated response by inducing the synthesis or altering expression of TLR-4 of SEQ ID NOS: 2, 4, 6, 98, and 99. Action, p. 6, ln. 18-20.

Claim 40 is directed to a screening method wherein a reporter gene is operatively positioned under the control of a nucleic acid segment comprising a promoter from a TLR-4 gene. In addition, claim 40 reads on a TLR-4 polypeptide that has the sequence of SEQ ID NOS: 2, 4, 6, 98, and 99. Appellants, therefore, submit that the enablement of claim 40 must be addressed separately.

Claims 55 and 56 are directed to screening methods wherein a putative modulator affects the transcription of TLR-4 and the translation of TLR-4, respectively. Appellants, therefore, submit that the enablement of claims 55 and 56 must be addressed separately.

8. Summary

The Action provides no factual basis or scientific principle upon which any doubt may be cast on the specification and the knowledge of those of skill in the art. The Action's unsupported allegations do not address the objective enablement provided by the specification and cannot carry the heavy burden placed upon the Examiner. Therefore, Appellants respectfully submit that the Examiner has not carried the burden of establishing a *prima facie* case of lack of enablement. *See In re Marzocchi,* 439 F.2d 220, 224, 169 USPQ 367, 370 (C.C.P.A. 1971); *In re Wright*, 999 F.2d 1557, 1561, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993). In view of the disclosure provided in the specification and the arguments provided above, Appellants request that the Board overturn the rejection.

D. Conclusion

It is respectfully submitted, in light of the above, that all claims are definite and enabled. Appellants, therefore, request that the Board overturn each of the pending grounds for rejection.

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Respectfully submitted,

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Date: October 23, 2003

APPENDIX 1 – PENDING CLAIMS WITH AMENDMENT TO CLAIM 67

38. A method of screening for modulators of a lipopolysaccharide mediated response comprising the steps of:

a) obtaining a cell expressing a TLR-4 polypeptide;

b) measuring a lipopolysaccharide mediated response mediated by the TLR-4 polypeptide;

c) contacting the TLR-4 polypeptide with a putative modulator;

d) assaying for a change in the lipopolysaccharide mediated response; and

e) comparing the lipopolysaccharide mediated responses mediated by the TLR-4 polypeptide obtained in steps b) and d) above

wherein a difference in the lipopolysaccharide mediated responses indicates that the putative modulator is a modulator of a lipopolysaccharide mediated response.

39. The method of claim 38, wherein the TLR-4 polypeptide has the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:98 or SEQ ID NO:99.

40. The method of claim 39, wherein the lipopolysaccharide mediated response mediated by the TLR-4 polypeptide is determined by determining the ability of the TLR-4 polypeptide to stimulate transcription of a reporter gene, the reporter gene operatively positioned under control of a nucleic acid segment comprising a promoter from a TLR-4 gene.

52. The method of claim 38, wherein said putative modulator is effective in altering the mediation of the lipopolysaccharide mediated response by TLR-4.

53. The method of claim 52, wherein said putative modulator is an agonist.

54. The method of claim 52, wherein said putative modulator is an antagonist.

55. The method of claim 52, wherein said putative modulator affects the transcription of TLR-4.

56. The method of claim 52, wherein said putative modulator affects the translation of TLR-4.

57. The method of claim 38, wherein the TLR-4 polypeptide has the amino acid sequence of SEQ ID NO:2.

58. The method of claim 38, wherein the TLR-4 polypeptide has the amino acid sequence of SEQ ID NO:4.

59. The method of claim 38, wherein the TLR-4 polypeptide has the amino acid sequence of SEQ ID NO:6.

60. The method of claim 38, wherein the TLR-4 polypeptide has the amino acid sequence of SEQ ID NO:98.

61. The method of claim 38, wherein the TLR-4 polypeptide has the amino acid sequence of SEQ ID NO:99.

63. The method of claim 38, wherein said putative modulator inhibits TLR-4 directed signaling of TNF secretion.

64. The method of claim 38, wherein said putative modulator stimulates TLR-4 directed signaling of TNF secretion.

65. The method of claim 38, wherein said putative modulator to be screened is obtained from a library of synthetic chemicals.

66. The method of claim 38, wherein said putative modulator to be screened is obtained from a natural source.

67. The method of claim 66, wherein said natural source is selected from the group consisting of animals, bacteria, fungi, plant sources and living marine samples.

68. The method of claim 38, wherein said putative modulator to be screened is a protein or peptide.

70. The method of claim 38, wherein said putative modulator to be screened is a nucleic acid molecule.

71. The method of claim 38, wherein said putative modulator to be screened is a stimulator of an immune response.

72. The method of claim 71, wherein said stimulator of an immune response is a cytokine.

73. The method of claim 71, wherein said stimulator of an immune response is an interferon.

74. The method of claim 38, wherein said TLR-4 polypeptide is encoded by a nucleic acid sequence selected from the group comprising SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:46, SEQ ID NO:47 and SEQ ID NO:48.

75. The method of claim 38, wherein said putative modulator to be screened is an IL-1 receptor antagonist.

100. The method of claim 38, wherein the TLR-4 polypeptide has the amino acid sequence selected from the group comprising SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:98 and SEQ ID NO:99.

101. The method of claim 38, wherein said putative modulator to be screened is a small molecule.

102. The method of claim 101, wherein said small molecule inhibits TLR-4 mediation of the lipopolysaccharide mediated response.

103. The method of claim 101, wherein said small molecule inhibits the lipopolysaccharide mediated response.

APPENDIX 2 – PENDING CLAIMS WITHOUT AMENDMENT TO CLAIM 67

38. A method of screening for modulators of a lipopolysaccharide mediated response comprising the steps of:

a) obtaining a cell expressing a TLR-4 polypeptide;

b) measuring a lipopolysaccharide mediated response mediated by the TLR-4 polypeptide;

c) contacting the TLR-4 polypeptide with a putative modulator;

d) assaying for a change in the lipopolysaccharide mediated response; and

e) comparing the lipopolysaccharide mediated responses mediated by the TLR-4 polypeptide obtained in steps b) and d) above

wherein a difference in the lipopolysaccharide mediated responses indicates that the putative modulator is a modulator of a lipopolysaccharide mediated response.

39. The method of claim 38, wherein the TLR-4 polypeptide has the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:98 or SEQ ID NO:99.

40. The method of claim 39, wherein the lipopolysaccharide mediated response mediated by the TLR-4 polypeptide is determined by determining the ability of the TLR-4 polypeptide to stimulate transcription of a reporter gene, the reporter gene operatively positioned under control of a nucleic acid segment comprising a promoter from a TLR-4 gene.

52. The method of claim 38, wherein said putative modulator is effective in altering the mediation of the lipopolysaccharide mediated response by TLR-4.

53. The method of claim 52, wherein said putative modulator is an agonist.

54. The method of claim 52, wherein said putative modulator is an antagonist.

55. The method of claim 52, wherein said putative modulator affects the transcription of TLR-4.

56. The method of claim 52, wherein said putative modulator affects the translation of TLR-4.

57. The method of claim 38, wherein the TLR-4 polypeptide has the amino acid sequence of SEQ ID NO:2.

58. The method of claim 38, wherein the TLR-4 polypeptide has the amino acid sequence of SEQ ID NO:4.

59. The method of claim 38, wherein the TLR-4 polypeptide has the amino acid sequence of SEQ ID NO:6.

60. The method of claim 38, wherein the TLR-4 polypeptide has the amino acid sequence of SEQ ID NO:98.

61. The method of claim 38, wherein the TLR-4 polypeptide has the amino acid sequence of SEQ ID NO:99.

63. The method of claim 38, wherein said putative modulator inhibits TLR-4 directed signaling of TNF secretion.

64. The method of claim 38, wherein said putative modulator stimulates TLR-4 directed signaling of TNF secretion.

65. The method of claim 38, wherein said putative modulator to be screened is obtained from a library of synthetic chemicals.

66. The method of claim 38, wherein said putative modulator to be screened is obtained from a natural source.

67. The method of claim 65, wherein said natural source is selected from the group consisting of animals, bacteria, fungi, plant sources and living marine samples.

68. The method of claim 38, wherein said putative modulator to be screened is a protein or peptide.

70. The method of claim 38, wherein said putative modulator to be screened is a nucleic acid molecule.

71. The method of claim 38, wherein said putative modulator to be screened is a stimulator of an immune response.

72. The method of claim 71, wherein said stimulator of an immune response is a cytokine.

73. The method of claim 71, wherein said stimulator of an immune response is an interferon.

74. The method of claim 38, wherein said TLR-4 polypeptide is encoded by a nucleic acid sequence selected from the group comprising SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:46, SEQ ID NO:47 and SEQ ID NO:48.

75. The method of claim 38, wherein said putative modulator to be screened is an IL-1 receptor antagonist.

100. The method of claim 38, wherein the TLR-4 polypeptide has the amino acid sequence selected from the group comprising SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:98 and SEQ ID NO:99.

101. The method of claim 38, wherein said putative modulator to be screened is a small molecule.

102. The method of claim 101, wherein said small molecule inhibits TLR-4 mediation of the lipopolysaccharide mediated response.

103. The method of claim 101, wherein said small molecule inhibits the lipopolysaccharide mediated response.

Exhibit 1

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Leukemia. 2003 Oct;17(10):2036-45.
PMID: 14513055 [PubMed - in process]

2: Sattler M, Pride YB, Ma P, Gramlich JL, Chu SC, Quinnan LA, Shirazian S, Liang C, Podar K, Christensen JG, Salgia R. Related Articles, Links

A novel small molecule met inhibitor induces apoptosis in cells transformed by the oncogenic TPR-MET tyrosine kinase.
Cancer Res. 2003 Sep 1;63(17):5462-9.
PMID: 14500382 [PubMed - in process]

3: Lin PF, Blair W, Wang T, Spicer T, Guo Q, Zhou N, Gong YF, Wang HG, Rose R, Yamanaka G, Robinson B, Li CB, Fridell R, Deminie C, Demers G, Yang Z, Zadjura L, Meanwell N, Colonno R. Related Articles, Links

A small molecule HIV-1 inhibitor that targets the HIV-1 envelope and inhibits CD4 receptor binding.
Proc Natl Acad Sci U S A. 2003 Sep 16;100(19):11013-8. Epub 2003 Aug 20.
PMID: 12930892 [PubMed - in process]

4: London CA, Hannah AL, Zadovoskaya R, Chien MB, Kollias-Baker C, Rosenberg M, Downing S, Post G, Boucher J, Shenoy N, Mendel DB, McMahon G, Cherrington JM. Related Articles, Links

Phase I dose-escalating study of SU11654, a small molecule receptor tyrosine kinase inhibitor, in dogs with spontaneous malignancies.
Clin Cancer Res. 2003 Jul;9(7):2755-68.
PMID: 12855656 [PubMed - in process]

5: Koehler AN, Shamji AF, Schreiber SL. Related Articles, Links

Discovery of an inhibitor of a transcription factor using small molecule microarrays and diversity-oriented synthesis.
J Am Chem Soc. 2003 Jul 16;125(28):8420-1. No abstract available.
PMID: 12848532 [PubMed - indexed for MEDLINE]

6: Suleymanov OD, Szalony JA, Salyers AK, LaChance RM, Parlow JJ, South MS, Wood RS, Nicholson NS. Related Articles, Links

Pharmacological interruption of acute thrombus formation with minimal hemorrhagic complications by a small molecule tissue factor/factor VIIa inhibitor: comparison to factor Xa and thrombin inhibition in a nonhuman primate thrombosis model.
J Pharmacol Exp Ther. 2003 Sep;306(3):1115-21. Epub 2003 Jun 26.
PMID: 12829728 [PubMed - indexed for MEDLINE]

☐ 7: Sun JP, Wu L, Fedorov AA, Almo SC, Zhang ZY. Related Articles, Links

Crystal structure of the Yersinia protein-tyrosine phosphatase YopH complexed with a specific small molecule inhibitor.
J Biol Chem. 2003 Aug 29;278(35):33392-9. Epub 2003 Jun 16.
PMID: 12810712 [PubMed - indexed for MEDLINE]

☐ 8: Patel N, Sun L, Moshinsky D, Chen H, Leahy KM, Le P, Moss KG, Wang X, Rice A, Tam D, Laird AD, Yu X, Zhang Q, Tang C, McMahon G, Howlett A. Related Articles, Links

A selective and oral small molecule inhibitor of vascular epithelial growth factor receptor (VEGFR)-2 and VEGFR-1 inhibits neovascularization and vascular permeability.
J Pharmacol Exp Ther. 2003 Sep;306(3):838-45. Epub 2003 May 23.
PMID: 12766257 [PubMed - indexed for MEDLINE]

☐ 9: Tian G, Ghanekar SV, Aharony D, Shenvi AB, Jacobs RT, Liu X, Greenberg BD. Related Articles, Links

The mechanism of gamma-secretase: multiple inhibitor binding sites for transition state analogs and small molecule inhibitors.
J Biol Chem. 2003 Aug 1;278(31):28968-75. Epub 2003 Apr 28.
PMID: 12719412 [PubMed - indexed for MEDLINE]

☐ 10: Ciardiello F, Caputo R, Damiano V, Caputo R, Troiani T, Vitagliano D, Carlomagno F, Veneziani BM, Fontanini G, Bianco AR, Tortora G. Related Articles, Links

Antitumor effects of ZD6474, a small molecule vascular endothelial growth factor receptor tyrosine kinase inhibitor, with additional activity against epidermal growth factor receptor tyrosine kinase.
Clin Cancer Res. 2003 Apr;9(4):1546-56.
PMID: 12684431 [PubMed - in process]

☐ 11: Todd B, Moore D, Deivanayagam CC, Lin GD, Chattopadhyay D, Maki M, Wang KK, Narayana SV. Related Articles, Links

A structural model for the inhibition of calpain by calpastatin: crystal structures of the native domain VI of calpain and its complexes with calpastatin peptide and a small molecule inhibitor.
J Mol Biol. 2003 Apr 18;328(1):131-46.
PMID: 12684003 [PubMed - indexed for MEDLINE]

☐ 12: Williams JA, Guicherit OM, Zaharian BI, Xu Y, Chai L, Wichterle H, Kon C, Gatchalian C, Porter JA, Rubin LL, Wang FY. Related Articles, Links

Identification of a small molecule inhibitor of the hedgehog signaling pathway: effects on basal cell carcinoma-like lesions.
Proc Natl Acad Sci U S A. 2003 Apr 15;100(8):4616-21. Epub 2003 Apr 04. Erratum in: Proc Natl Acad Sci U S A. 2003 Jul 8;100(14):8607.
PMID: 12679522 [PubMed - indexed for MEDLINE]

☐ 13: Haggarty SJ, Koeller KM, Wong JC, Grozinger CM, Schreiber SL. Related Articles, Links

Domain-selective small-molecule inhibitor of histone deacetylase 6 (HDAC6)-mediated tubulin deacetylation.
Proc Natl Acad Sci U S A. 2003 Apr 15;100(8):4389-94. Epub 2003 Apr 03.
PMID: 12677000 [PubMed - indexed for MEDLINE]

☐ 14: Giles FJ, Cooper MA, Silverman L, Karp JE, Lancet JE, Zangari M, Shami PJ, Khan KD, Hannah AL, Cherrington JM, Thomas DA, Garcia-Manero G, Albitar M, Kantarjian HM, Stopeck AT. Related Articles, Links

Phase II study of SU5416--a small-molecule, vascular endothelial growth factor

tyrosine-kinase receptor inhibitor--in patients with refractory myeloproliferative diseases.
Cancer. 2003 Apr 15;97(8):1920-8.
PMID: 12673719 [PubMed - indexed for MEDLINE]

☐ 15: Braisted AC, Oslob JD, Delano WL, Hyde J, McDowell RS, Waal N, Yu C, Arkin MR, Raimundo BC. Related Articles, Links

Discovery of a potent small molecule IL-2 inhibitor through fragment assembly.
J Am Chem Soc. 2003 Apr 2;125(13):3714-5. No abstract available.
PMID: 12656598 [PubMed - indexed for MEDLINE]

☐ 16: Giles FJ, Stopeck AT, Silverman LR, Lancet JE, Cooper MA, Hannah AL, Cherrington JM, O'Farrell AM, Yuen HA, Louie SG, Hong W, Cortes JE, Verstovsek S, Albitar M, O'Brien SM, Kantarjian HM, Karp JE. Related Articles, Links

SU5416, a small molecule tyrosine kinase receptor inhibitor, has biologic activity in patients with refractory acute myeloid leukemia or myelodysplastic syndromes.
Blood. 2003 Aug 1;102(3):795-801. Epub 2003 Mar 20.
PMID: 12649163 [PubMed - indexed for MEDLINE]

☐ 17: Daines RA, Pendrak I, Sham K, Van Aller GS, Konstantinidis AK, Lonsdale JT, Janson CA, Qiu X, Brandt M, Khandekar SS, Silverman C, Head MS. Related Articles, Links

First X-ray cocrystal structure of a bacterial FabH condensing enzyme and a small molecule inhibitor achieved using rational design and homology modeling.
J Med Chem. 2003 Jan 2;46(1):5-8.
PMID: 12502353 [PubMed - indexed for MEDLINE]

☐ 18: Muljo SA, Schlissel MS. Related Articles, Links

A small molecule Abl kinase inhibitor induces differentiation of Abelson virus-transformed pre-B cell lines.
Nat Immunol. 2003 Jan;4(1):31-7. Epub 2002 Dec 02.
PMID: 12469118 [PubMed - indexed for MEDLINE]

☐ 19: Piraino PS, Yednock TA, Freedman SB, Messersmith EK, Pleiss MA, Vandevert C, Thorsett ED, Karlik SJ. Related Articles, Links

Prolonged reversal of chronic experimental allergic encephalomyelitis using a small molecule inhibitor of alpha4 integrin.
J Neuroimmunol. 2002 Oct;131(1-2):147-59.
PMID: 12458046 [PubMed - indexed for MEDLINE]

☐ 20: Greenbaum DC, Arnold WD, Lu F, Hayrapetian L, Baruch A, Krumrine J, Toba S, Chehade K, Bromme D, Kuntz ID, Bogyo M. Related Articles, Links

Small molecule affinity fingerprinting. A tool for enzyme family subclassification, target identification, and inhibitor design.
Chem Biol. 2002 Oct;9(10):1085-94.
PMID: 12401493 [PubMed - indexed for MEDLINE]

☐ 21: Henderson WR Jr, Chi EY, Teo JL, Nguyen C, Kahn M. Related Articles, Links

A small molecule inhibitor of redox-regulated NF-kappa B and activator protein-1 transcription blocks allergic airway inflammation in a mouse asthma model.
J Immunol. 2002 Nov 1;169(9):5294-9.
PMID: 12391249 [PubMed - indexed for MEDLINE]

☐ 22: Waegell W, Babineau M, Hart M, Dixon K, McRae B, Wallace C, Leach M, Ratnofsky S, Belanger A, Hirst G, Rossini A, Appel M, Mordes J, Greiner D, Banerjee S. Related Articles, Links

A420983, a novel, small molecule inhibitor of LCK prevents allograft rejection.
Transplant Proc. 2002 Aug;34(5):1411-7. No abstract available.
PMID: 12176420 [PubMed - indexed for MEDLINE]

23: Anaya-Prado R, Ramos-Kelly JR, Toledo-Pereyra LH, Walsh J, Ward PA. Related Articles, Links

Multiple selectin blockade with a small-molecule selectin inhibitor does not affect survival after a second inflammatory challenge with nonlethal LPS.
J Invest Surg. 2002 May-Jun;15(3):171-80.
PMID: 12139789 [PubMed - indexed for MEDLINE]

24: Weisberg E, Boulton C, Kelly LM, Manley P, Fabbro D, Meyer T, Gilliland DG, Griffin JD. Related Articles, Links

Inhibition of mutant FLT3 receptors in leukemia cells by the small molecule tyrosine kinase inhibitor PKC412.
Cancer Cell. 2002 Jun;1(5):433-43.
PMID: 12124173 [PubMed - indexed for MEDLINE]

25: Kao RY, Jenkins JL, Olson KA, Key ME, Fett JW, Shapiro R. Related Articles, Links

A small-molecule inhibitor of the ribonucleolytic activity of human angiogenin that possesses antitumor activity.
Proc Natl Acad Sci U S A. 2002 Jul 23;99(15):10066-71. Epub 2002 Jul 12.
PMID: 12118120 [PubMed - indexed for MEDLINE]

26: Kipp RA, Case MA, Wist AD, Cresson CM, Carrell M, Griner E, Wiita A, Albiniak PA, Chai J, Shi Y, Semmelhack MF, McLendon GL. Related Articles, Links

Molecular targeting of inhibitor of apoptosis proteins based on small molecule mimics of natural binding partners.
Biochemistry. 2002 Jun 11;41(23):7344-9.
PMID: 12044166 [PubMed - indexed for MEDLINE]

27: Mattsson C, Bjorkman JA, Abrahamsson T, Nerme V, Schatteman K, Leurs J, Scharpe S, Hendriks D. Related Articles, Links

Local proCPU (TAFI) activation during thrombolytic treatment in a dog model of coronary artery thrombosis can be inhibited with a direct, small molecule thrombin inhibitor (melagatran).
Thromb Haemost. 2002 Apr;87(4):557-62.
PMID: 12008935 [PubMed - indexed for MEDLINE]

28: Lark MW, Stroup GB, James IE, Dodds RA, Hwang SM, Blake SM, Lechowska BA, Hoffman SJ, Smith BR, Kapadia R, Liang X, Erhard K, Ru Y, Dong X, Marquis RW, Veber D, Gowen M. Related Articles, Links

A potent small molecule, nonpeptide inhibitor of cathepsin K (SB 331750) prevents bone matrix resorption in the ovariectomized rat.
Bone. 2002 May;30(5):746-53.
PMID: 11996914 [PubMed - indexed for MEDLINE]

29: Gowan SM, Harrison JR, Patterson L, Valenti M, Read MA, Neidle S, Kelland LR. Related Articles, Links

A G-quadruplex-interactive potent small-molecule inhibitor of telomerase exhibiting in vitro and in vivo antitumor activity.
Mol Pharmacol. 2002 May;61(5):1154-62.
PMID: 11961134 [PubMed - indexed for MEDLINE]

30: Swinney DC, Xu YZ, Scarafia LE, Lee I, Mak AY, Gan QF, Ramesha CS, Mulkins MA, Dunn J, So OY, Biegel T, Dinh M, Volkel P, Barnett J, Dalrymple SA, Lee S, Huber M. Related Articles, Links

A small molecule ubiquitination inhibitor blocks NF-kappa B-dependent cytokine expression in cells and rats.
J Biol Chem. 2002 Jun 28;277(26):23573-81. Epub 2002 Apr 11.
PMID: 11950839 [PubMed - indexed for MEDLINE]

☐ 31: Kappler F, Schwartz ML, Su B, Tobia AM, Brown T. Related Articles, Links

DYN 12, a small molecule inhibitor of the enzyme amadorase, lowers plasma 3-deoxyglucosone levels in diabetic rats.
Diabetes Technol Ther. 2001 Winter;3(4):609-16.
PMID: 11911173 [PubMed - indexed for MEDLINE]

☐ 32: Folkes A, Brown SD, Canne LE, Chan J, Engelhardt E, Epshteyn S, Faint R, Golec J, Hanel A, Kearney P, Leahy JW, Mac M, Matthews D, Prisbylla MP, Sanderson J, Simon RJ, Tesfai Z, Vicker N, Wang S, Webb RR, Charlton P. Related Articles, Links

Design, synthesis and in vitro evaluation of potent, novel, small molecule inhibitors of plasminogen activator inhibitor-1.
Bioorg Med Chem Lett. 2002 Apr 8;12(7):1063-6.
PMID: 11909718 [PubMed - indexed for MEDLINE]

☐ 33: Trkola A, Kuhmann SE, Strizki JM, Maxwell E, Ketas T, Morgan T, Pugach P, Xu S, Wojcik L, Tagat J, Palani A, Shapiro S, Clader JW, McCombie S, Reyes GR, Baroudy BM, Moore JP. Related Articles, Links

HIV-1 escape from a small molecule, CCR5-specific entry inhibitor does not involve CXCR4 use.
Proc Natl Acad Sci U S A. 2002 Jan 8;99(1):395-400.
PMID: 11782552 [PubMed - indexed for MEDLINE]

☐ 34: Bedalov A, Gatbonton T, Irvine WP, Gottschling DE, Simon JA. Related Articles, Links

Identification of a small molecule inhibitor of Sir2p.
Proc Natl Acad Sci U S A. 2001 Dec 18;98(26):15113-8.
PMID: 11752457 [PubMed - indexed for MEDLINE]

☐ 35: Cheung A, Dantzig JA, Hollingworth S, Baylor SM, Goldman YE, Mitchison TJ, Straight AF. Related Articles, Links

A small-molecule inhibitor of skeletal muscle myosin II.
Nat Cell Biol. 2002 Jan;4(1):83-8.
PMID: 11744924 [PubMed - indexed for MEDLINE]

☐ 36: Thurmond RL, Wadsworth SA, Schafer PH, Zivin RA, Siekierka JJ. Related Articles, Links

Kinetics of small molecule inhibitor binding to p38 kinase.
Eur J Biochem. 2001 Nov;268(22):5747-54.
PMID: 11722559 [PubMed - indexed for MEDLINE]

☐ 37: Buerke M, Schwertz H, Seitz W, Meyer J, Darius H. Related Articles, Links

Novel small molecule inhibitor of C1s exerts cardioprotective effects in ischemia-reperfusion injury in rabbits.
J Immunol. 2001 Nov 1;167(9):5375-80.
PMID: 11673555 [PubMed - indexed for MEDLINE]

☐ 38: Strizki JM, Xu S, Wagner NE, Wojcik L, Liu J, Hou Y, Endres M, Palani A, Shapiro S, Clader JW, Greenlee WJ, Tagat JR, McCombie S, Cox K, Fawzi AB, Chou CC, Pugliese-Sivo C, Davies L, Moreno ME, Ho DD, Trkola A, Stoddart CA, Moore JP, Reyes GR, Baroudy BM. Related Articles, Links

SCH-C (SCH 351125), an orally bioavailable, small molecule antagonist of the chemokine receptor CCR5, is a potent inhibitor of HIV-1 infection in vitro and in

vivo.
Proc Natl Acad Sci U S A. 2001 Oct 23;98(22):12718-23. Epub 2001 Oct 16.
PMID: 11606733 [PubMed - indexed for MEDLINE]

☐ **39:** Soni R, O'Reilly T, Furet P, Muller L, Stephan C, Zumstein-Mecker S, Fretz H, Fabbro D, Chaudhuri B. Related Articles, Links

Selective in vivo and in vitro effects of a small molecule inhibitor of cyclin-dependent kinase 4.
J Natl Cancer Inst. 2001 Mar 21;93(6):436-46.
PMID: 11259469 [PubMed - indexed for MEDLINE]

☐ **40:** [No authors listed] Related Articles, Links

A small-molecule PDGF inhibitor
Nat Biotechnol. 2000 Oct;18(10):1026. No abstract available.
PMID: 11017013 [PubMed - as supplied by publisher]

☐ **41:** Glushakova S, Dubrovsky L, Grivel J, Haffar O, Bukrinsky M. Related Articles, Links

Small molecule inhibitor of HIV-1 nuclear import suppresses HIV-1 replication in human lymphoid tissue ex vivo: a potential addition to current anti-HIV drug repertoire.
Antiviral Res. 2000 Aug;47(2):89-95.
PMID: 10996396 [PubMed - indexed for MEDLINE]

☐ **42:** Kapoor TM, Mayer TU, Coughlin ML, Mitchison TJ. Related Articles, Links

Probing spindle assembly mechanisms with monastrol, a small molecule inhibitor of the mitotic kinesin, Eg5.
J Cell Biol. 2000 Sep 4;150(5):975-88.
PMID: 10973989 [PubMed - indexed for MEDLINE]

☐ **43:** Abraham WM, Gill A, Ahmed A, Sielczak MW, Lauredo IT, Botinnikova Y, Lin KC, Pepinsky B, Leone DR, Lobb RR, Adams SP. Related Articles, Links

A small-molecule, tight-binding inhibitor of the integrin alpha(4)beta(1) blocks antigen-induced airway responses and inflammation in experimental asthma in sheep.
Am J Respir Crit Care Med. 2000 Aug;162(2 Pt 1):603-11.
PMID: 10934094 [PubMed - indexed for MEDLINE]

☐ **44:** Ramos-Kelly JR, Toledo-Pereyra LH, Jordan J, Rivera-Chavez F, Rohs T, Holevar M, Dixon RA, Yun E, Ward PA. Related Articles, Links

Multiple selectin blockade with a small molecule inhibitor downregulates liver chemokine expression and neutrophil infiltration after hemorrhagic shock.
J Trauma. 2000 Jul;49(1):92-100.
PMID: 10912864 [PubMed - indexed for MEDLINE]

☐ **45:** Mendel DB, Laird AD, Smolich BD, Blake RA, Liang C, Hannah AL, Shaheen RM, Ellis LM, Weitman S, Shawver LK, Cherrington JM. Related Articles, Links

Development of SU5416, a selective small molecule inhibitor of VEGF receptor tyrosine kinase activity, as an anti-angiogenesis agent.
Anticancer Drug Des. 2000 Feb;15(1):29-41. Review.
PMID: 10888034 [PubMed - indexed for MEDLINE]

☐ **46:** Dragic T, Trkola A, Thompson DA, Cormier EG, Kajumo FA, Maxwell E, Lin SW, Ying W, Smith SO, Sakmar TP, Moore JP. Related Articles, Links

A binding pocket for a small molecule inhibitor of HIV-1 entry within the transmembrane helices of CCR5.
Proc Natl Acad Sci U S A. 2000 May 9;97(10):5639-44.

PMID: 10779565 [PubMed - indexed for MEDLINE]

☐ **47:** Katz BA, Mackman R, Luong C, Radika K, Martelli A, Sprengeler PA, Wang J, Chan H, Wong L. Related Articles, Links

Structural basis for selectivity of a small molecule, S1-binding, submicromolar inhibitor of urokinase-type plasminogen activator.
Chem Biol. 2000 Apr;7(4):299-312.
PMID: 10779411 [PubMed - indexed for MEDLINE]

☐ **48:** Mondal MS, Wang Z, Seeds AM, Rando RR. Related Articles, Links

The specific binding of small molecule isoprenoids to rhoGDP dissociation inhibitor (rhoGDI).
Biochemistry. 2000 Jan 18;39(2):406-12.
PMID: 10631002 [PubMed - indexed for MEDLINE]

☐ **49:** Ramos-Kelly JR, Toledo-Pereyra LH, Jordan JA, Rivera-Chavez FA, Dixon RA, Ward PA. Related Articles, Links

Upregulation of lung chemokines associated with hemorrhage is reversed with a small molecule multiple selectin inhibitor.
J Am Coll Surg. 1999 Dec;189(6):546-53.
PMID: 10589590 [PubMed - indexed for MEDLINE]

☐ **50:** Mayer TU, Kapoor TM, Haggarty SJ, King RW, Schreiber SL, Mitchison TJ. Related Articles, Links

Small molecule inhibitor of mitotic spindle bipolarity identified in a phenotype-based screen.
Science. 1999 Oct 29;286(5441):971-4.
PMID: 10542155 [PubMed - indexed for MEDLINE]

☐ **51:** Arakaki R, Tamamura H, Premanathan M, Kanbara K, Ramanan S, Mochizuki K, Baba M, Fujii N, Nakashima H. Related Articles, Links

T134, a small-molecule CXCR4 inhibitor, has no cross-drug resistance with AMD3100, a CXCR4 antagonist with a different structure.
J Virol. 1999 Feb;73(2):1719-23.
PMID: 9882387 [PubMed - indexed for MEDLINE]

☐ **52:** Nishio M, Watanabe Y, Hidaka H. Related Articles, Links

HMN-1180, a small molecule inhibitor of neuronal nitric oxide synthase.
J Pharmacol Exp Ther. 1998 Dec;287(3):1063-7.
PMID: 9864293 [PubMed - indexed for MEDLINE]

☐ **53:** Howard OM, Korte T, Tarasova NI, Grimm M, Turpin JA, Rice WG, Michejda CJ, Blumenthal R, Oppenheim JJ. Related Articles, Links

Small molecule inhibitor of HIV-1 cell fusion blocks chemokine receptor-mediated function.
J Leukoc Biol. 1998 Jul;64(1):6-13. Review.
PMID: 9665268 [PubMed - indexed for MEDLINE]

☐ **54:** Hursting MJ, Alford KL, Becker JC, Brooks RL, Joffrion JL, Knappenberger GD, Kogan PW, Kogan TP, McKinney AA, Schwarz RP Jr. Related Articles, Links

Novastan (brand of argatroban): a small-molecule, direct thrombin inhibitor.
Semin Thromb Hemost. 1997;23(6):503-16. Review.
PMID: 9469622 [PubMed - indexed for MEDLINE]

☐ **55:** Donzella GA, Schols D, Lin SW, Este JA, Nagashima KA, Maddon PJ, Allaway GP, Sakmar TP, Henson G, De Clercq E, Moore JP. Related Articles, Links

AMD3100, a small molecule inhibitor of HIV-1 entry via the CXCR4 co-receptor.
Nat Med. 1998 Jan;4(1):72-7.
PMID: 9427609 [PubMed - indexed for MEDLINE]

☐ 56: Palma-Vargas JM, Toledo-Pereyra L, Dean RE, Harkema JM, Dixon RA, Kogan TP. Related Articles, Links

Small-molecule selectin inhibitor protects against liver inflammatory response after ischemia and reperfusion.
J Am Coll Surg. 1997 Oct;185(4):365-72.
PMID: 9328385 [PubMed - indexed for MEDLINE]

☐ 57: Doranz BJ, Grovit-Ferbas K, Sharron MP, Mao SH, Goetz MB, Daar ES, Doms RW, O'Brien WA. Related Articles, Links

A small-molecule inhibitor directed against the chemokine receptor CXCR4 prevents its use as an HIV-1 coreceptor.
J Exp Med. 1997 Oct 20;186(8):1395-400.
PMID: 9334380 [PubMed - indexed for MEDLINE]

☐ 58: Murakami T, Nakajima T, Koyanagi Y, Tachibana K, Fujii N, Tamamura H, Yoshida N, Waki M, Matsumoto A, Yoshie O, Kishimoto T, Yamamoto N, Nagasawa T. Related Articles, Links

A small molecule CXCR4 inhibitor that blocks T cell line-tropic HIV-1 infection.
J Exp Med. 1997 Oct 20;186(8):1389-93.
PMID: 9334379 [PubMed - indexed for MEDLINE]

☐ 59: Abraham WM, Ahmed A, Sielczak MW, Narita M, Arrhenius T, Elices MJ. Related Articles, Links

Blockade of late-phase airway responses and airway hyperresponsiveness in allergic sheep with a small-molecule peptide inhibitor of VLA-4.
Am J Respir Crit Care Med. 1997 Sep;156(3 Pt 1):696-703.
PMID: 9309981 [PubMed - indexed for MEDLINE]

☐ 60: Burke TR Jr, Ye B, Yan X, Wang S, Jia Z, Chen L, Zhang ZY, Barford D. Related Articles, Links

Small molecule interactions with protein-tyrosine phosphatase PTP1B and their use in inhibitor design.
Biochemistry. 1996 Dec 17;35(50):15989-96.
PMID: 8973169 [PubMed - indexed for MEDLINE]

☐ 61: Safrany ST, Sawyer DA, Nahorski SR, Potter BV. Related Articles, Links

Synthetic D- and L-enantiomers of 2,2-difluoro-2-deoxy-myo-inositol 1,4,5-trisphosphate interact differently with myo-inositol 1,4,5-trisphosphate binding proteins: identification of a potent small molecule 3-kinase inhibitor.
Chirality. 1992;4(7):415-22.
PMID: 1334423 [PubMed - indexed for MEDLINE]

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Exhibit 2

CURRICULUM VITAE

Name:	David Dunbar Chaplin
Date of Birth:	August 28, 1952
Place of Birth:	London, England
Citizenship:	U.S.A.
Social Security Number:	067-38-2393
Home Address:	406 Wildwood Lane Indian Springs, AL 35124
Current Position:	Charles H. McCauley Professor and Chair Department of Microbiology University of Alabama at Birmingham 845 19th Street South, BBRB 276/11 Birmingham, AL 35294-2170
Undergraduate Education:	Harvard College Cambridge, Massachusetts A.B. June, 1973
Graduate Education:	Washington University St. Louis, Missouri M.D. May, 1980 Ph.D. May, 1980

Post-doctoral Training:

1982-1984	Harvard Medical School, Department of Genetics Boston, Massachusetts, Fellow
1980-1982	University of Texas, Southwestern Medical School Parkland Memorial Hospital, Dallas, Texas Internal Medicine Residency

Academic Appointments:

2001-present	Chairman, University of Alabama at Birmingham, Department of Microbiology, Birmingham, AL
2001-present	Senior Scientist, Comprehensive Cancer Center, University of Alabama at Birmingham
1995-2001	Associate Physician, Barnes-Jewish Hospital, University of Washington, St. Louis, MO
1995-2001	Professor, Washington University School of Medicine, Departments of Medicine, Genetics, and Molecular Microbiology, St. Louis, MO
1994-2001	Chief, Div. of Allergy and Immunology, Washington University School of Medicine, Department of Medicine

Academic Appointments (continued):

1992-1995	Assoc. Professor, Washington University School of Medicine, Department of Genetics, St. Louis, MO
1991-1995	Assoc. Professor, Washington University School of Medicine, Department of Medicine and Molecular Microbiology, St. Louis, MO
1989-1992	Asst. Professor, Washington University School of Medicine, Department of Genetics, St. Louis, MO
1984-1995	Assistant Physician, Barnes-Jewish Hospital, University of Washington, St. Louis, MO
1984-2001	Assoc. Investigator, Howard Hughes Medical Institute
1984-1991	Asst. Professor, Washington University School of Medicine, Dept. of Medicine and Molecular Microbiology, St. Louis, MO

Honors/Awards:

2001	Fellow, American Academy of Allergy, Asthma and Immunology
1997	Association of American Physicians
1995-1998	Councilor, American Society for Clinical Investigation
1993	Fellow, American Association for the Advancement of Science
1993	American Society for Clinical Investigation
1982-1984	Jane Coffin Childs Memorial Fund for Medical Research Fellowship
1980	Alpha Omega Alpha
1974-1980	Medical Scientist Trainee

Scientific Organizations:

2001-present	Secretary, American Academy of Allergy, Asthma and Immunology, Basic and Clinical Immunology Interest Section,
1994-2001	Associate Editor, Journal of Immunology
1993-present	International Cytokine Society
1991-present	American Academy of Allergy, Asthma and Immunology
1991-1996	Associate Editor, Diabetes
1989-1991	Associate Editor, The New Biologist
1989-present	American Society of Human Genetics
1986-present	American Association of Immunologists
1985-present	American Federation of Clinical Research
1984-present	American Association for the Advancement of Science

Keywords: Inflammatory Cytokines; TNF; IL-1; Asthma Pathogenesis; Lymphoid Tissue Development; Th Cell Function; Germinal Centers; Follicular Dendritic Cells

Publications:

1. Feagler, J.R., Tillack, T.W., Chaplin, D.D., and Majerus, P.W. (1974): The effects of thrombin on phytohemagglutinin receptor sites in human platelets. *J Cell Biol* 60:541-553.

2. Wedner, H.J., Chaplin, D.D., and Parker, C.W. (1977): Evidence for an early sulfhydryl reagent sensitive step during lymphocyte activation. In: Regulatory Mechanisms in Lymphocyte Activation, edited by D.O. Lucas, pp. 456-458, Academic Press, New York.

3. Chaplin, D.D., and Wedner, H.J. (1978): Inhibition of lectin-induced lymphocyte activation by diamide and other sulfhydryl reagents. *Cell Immunol* 36:303-311.

4. Chaplin, D.D., Wedner, H.J., and Parker, C.W. (1979): Protein phosphorylation in human peripheral blood lymphocytes: Subcellular distribution and partial characterization of adenosine 3', 5'-monophosphate-dependent protein kinase. *Biochem J* 182:525-536.

5. Chaplin, D.D., Wedner, H.J., and Parker, C.W. (1979): Protein phosphorylation in human peripheral blood lymphocytes: Phosphorylation of endogenous plasma membrane and cytoplasmic proteins. *Biochem J* 182:537-546.

6. Chaplin, D.D., Wedner, H.J., and Parker, C.W. (1980): Protein phosphorylation in human peripheral blood lymphocytes: Mitogen-induced increases in protein phosphorylation in intact lymphocytes. *J Immunol* 124:2390-2398.

7. Chaplin, D.D., Wedner, H.J., and Parker, C.W. (1980): Protein phosphorylation and lymphocyte activation. In: Biological Basis of Immunodeficiency, edited by E.W. Gelfand and H.-M. Dosch, pp. 269-281, Raven Press, New York.

8. Udey, M.C., Chaplin, D.D., Wedner, H.J., and Parker, C.W. (1980): Early activation events in lectin-stimulated human lymphocytes: Evidence that wheat germ agglutinin and mitogenic lectins cause similar early changes in lymphocyte metabolism. *J Immunol* 125:1544-1550.

9. Chaplin, D.D., Woods, D.E., Whitehead, A.S., Goldberger, G., Colten, H.R., and Seidman, J.G. (1983): Molecular map of the murine S region. *Proc Natl Acad Sci U S A* 80:6947-6951.

10. Chaplin, D.D., Sackstein, R., Perlmutter, D.H., Weis, J.H., Kruse, T.A., Coligan, J., Colten, H.R., and Seidman, J.G. (1984): Expression of hemolytically active murine fourth component of complement in transfected L cells. *Cell* 37:569-576.

11. Buse, J.B., Chaplin, D.D., Ben-Nun, A., Klein, K.A., Eisenbarth, G.S., Seidman, J.G., and Jackson, R.A. (1984): Class I, II, and III major histocompatibility complex gene polymorphisms in BB rats. *Diabetalogia* 27:77-79.

12. Weis, J.H., Nelson, D.L., Przyborski, M.J., Chaplin, D.D., Mulligan, R.C., Housman, D.E., and Seidman, J.G. (1984): Eukaryotic chromosome transfer: Linkage of the murine major histocompatibility complex to an inserted dominant selectable marker. *Proc Natl Acad Sci U S A* 81:4879-4884.

13. White, P.C., Chaplin, D.D., Weis, J.H., Dupont, B., New, M.I., and Seidman, J.G. (1984): Two steroid 21-hydroxylase genes are located in the murine S region. *Nature* 312:465-467.

14. White, P.C., Grossberger, D., Onufer, B., Chaplin, D.D., New, M.I., Dupont, B., and Strominger, J.L. (1985): Two genes encoding steroid 21-hydroxylase are located near the genes encoding the fourth component of complement in man. *Proc Natl Acad Sci U S A* 82:1089-1093.

15. Perlmutter, D.H., Colten, H.R., Grossberger, D., Strominger, J.L., Seidman, J.G., and Chaplin, D.D. (1985): Expression of complement proteins C2 and Factor B in transfected L cells. *J Clin Invest* 76:1449-1454.

16. Chaplin, D.D. (1985): Molecular organization and in vitro expression of murine class III genes. *Immunol Rev* 87:61-80.

17. Parker, K.L., Chaplin, D.D., Wong, M., Seidman, J.G., Smith, J.A., and Schimmer, B.P. (1985): Expression of murine 21-hydroxylase in mouse adrenal glands and in transfected Y1 adrenocortical tumor cells. *Proc Natl Acad Sci U S A* 82:7860-7864.

18. Buse, J.B., Rifai-Haddad, R., Lees, S., Taniguchi, H., Chaplin, D.D., Milford, E.M., Seidman, J.G., Eisenbarth, G.S., and Jackson, R.A. (1985): Major histocompatibility complex restriction fragment length polymorphisms define three diabetogenic haplotypes in BB and BBN rats. *J Exp Med* 162:444-458.

19. Chaplin, D.D., Galbraith, L.G., Seidman, J.G., White, P.C., and Parker, K.L. (1986): Nucleotide sequence analysis of murine 21-hydroxylase genes: mutations affecting gene expression. *Proc Natl Acad Sci U S A* 83:9601-9605.

20. Parker, K.L., Chaplin, D.D., Wong, M., Seidman, J.G., and Schimmer, B.P. (1986): Molecular analysis of 21-hydroxylase gene expression in mouse adrenal cells. *Endocr Res* 12:409-427.

21. Parker, K.L., Schimmer, B.P., Chaplin, D.D., and Seidman, J.G. (1986): Characterization of a regulatory region of the steroid 21-hydroxylase gene. *J Biol Chem* 261:15353-15355.

22. Hansbrough, J.R., Wedner, H.J., and Chaplin, D.D. (1987): Anaphylaxis to intravenous furosemide. *J Allergy Clin Immunol* 80:538-541.

23. Holers, V.M., Chaplin, D.D., Leykam, J.F., Gruner, B.A., Kumar, V., and Atkinson, J.P. (1987): Human complement C3b/C4b receptor (CR1) mRNA polymorphism that correlates with the CR1 allelic molecular weight polymorphism. *Proc Natl Acad Sci U S A* 84:2459-2463.

24. Graham, M.Y., Otani, T., Boime, I., Olson, M.V., Carle, G.F., and Chaplin, D.D. (1987): Cosmid mapping of the human chorionic gonadotropin β subunit genes by field-inversion gel electrophoresis. *Nucleic Acids Res* 15:4437-4448.

25. Fuhlbrigge, R.C., Chaplin, D.D., Kiely, J.-M., and Unanue, E.R. (1987): Regulation of interleukin 1 gene expression by adherence and lipopolysaccharide. *J Immunol* 138:3799-3802.

26. Unanue, E.R., Weaver, C.T., Fuhlbrigge, R.C., Kiely, J.-M., Chaplin, D.D.(1987): Membrane IL-1: a key protein in antigen presentation. *Annales de l'Institut Pasteur/Immunology* 138:489-492.

27. D'Eustachio, P., Jadidi, S., Fuhlbrigge, R.C., Gray, P.W., and Chaplin, D.D. (1987): Interleukin-1 α and β genes: linkage on chromosome 2 in the mouse. *Immunogenetics* 26:339-343.

28. Kemp, M.E., Atkinson, J.P., Skanes, V.M., Levine, R.P., and Chaplin, D.D. (1987): Deletion of C4A genes in patients with systemic lupus erythematosus. *Arthritis Rheum* 30:1015-1022.

29. Chou, H.S., Nelson, C.A., Godambe, S.A., Chaplin, D.D., and Loh, D.Y. (1987): Germline organization of the murine T-cell receptor β-chain genes. *Science* 238:545-548.

30. Chaplin, D.D., and Kemp, M.E. (1987): The major histocompatibility complex and autoimmunity. In, The Year in Immunology, 1986/87, vol. 3, ed. Cruse, J.M., Karger, Basel, pp. 179-198.

31. Chaplin, D.D., Fuhlbrigge, R.C., Jadidi, S., Sheehan, K.C.F., Schreiber, R.D., Gray, P.W., Unanue, E.R., and D'Eustachio, P. (1988): Restriction fragment length polymorphisms and linkage of murine IL-1 α and β genes on chromosome 2. In, Progress in Leukocyte Biology, Vol. 8, "Monokines and Other Non-lymphocytic Cytokines" eds. Powanda, M.C., Oppenheim, J.J., Kluger, M.J., and Dinarello, C.A., Alan R. Liss, New York, pp. 41-46.

32. Otani, T., Otani, F., Krych, M., Chaplin, D.D., and Boime, I. (1988): Identification of a promoter region in the CGβ gene cluster. *J Biol Chem* 263:7322-7329.

33. Fuhlbrigge, R.C., Sheehan, K.C.F., Schreiber, R.D., Chaplin, D.D., and Unanue, E.R. (1988): Monoclonal antibodies to murine interleukin-1α: production, characterization, and inhibition of membrane-associated interleukin-1 activity. *J Immunol* 141:2643-2650.

34. Fuhlbrigge, R.C., Fine, S.M., Unanue, E.R., and Chaplin, D.D. (1988): Expression of membrane interleukin-1 by fibroblasts transfected with murine pro-interleukin-1α cDNA. *Proc Natl Acad Sci U S A* 85:5649-5653.

35. Vik, D.P., Keeney, J.B., Munoz-Canoves, P., Chaplin, D.D., and Tack, B.F. (1988): Structure of the murine complement factor H gene. *J Biol Chem* 263:16720-16724.

36. Fuhlbrigge, R.C., Hogquist, K.A., Unanue, E.R., and Chaplin, D.D. (1989): Molecular Biology and Genetics of Interleukin-1. In, The Year in Immunology, 1988, vol. 5, ed. Cruse, J.M. and Lewis, R.E., Karger, Basel, pp. 21-37.

37. Barnum, S.R., Kristensen, T., Chaplin, D.D., Seldin, M.F., and Tack, B.F. (1989): Molecular analysis of the murine C4b-binding protein gene. Chromosome assignment and partial gene organization. *Biochemistry* 28:8312-8317.

38. Storb, U., Engler, P., Hagman, J., Gollahon, K., Manz, J., Roth, P., Rudin, C., Doglio, L., Hackett, J., Haasch, D., Chaplin, D.D., Lo, D., and Brinster, R. (1989): Control of expression of immunoglobulin genes. *Progress in Immunology* 7:316-323.

39. Vik, D.P., Munoz-Canoves, P., Chaplin, D.D., and Tack, B.F. (1989): Factor H. *Curr Top Microbiol Immunol* 153:147-162.

40. Boime, I., Otani, T., Otani, F., and Chaplin, D.D. (1989): Regulation of the chorionic gonadotrophin β subunit genes. *Reprod Fertil Suppl* 37:1-10.

41. Amiguet, P., D'Eustachio, P., Kristensen, T., Wetsel, R.A., Saris, C.J.M., Hunter, T., Chaplin, D.D., and Tack, B.F. (1990): Structure and chromosome assignment of the murine p36 (calpactin-I heavy-chain) gene. *Biochemistry* 29:1226-1232.

42. Vik, D.P., Munoz-Canoves, P., Kozono, H., Martin, L.G., Tack, B.F., and Chaplin, D.D. (1990): Identification and sequence analysis of four complement factor H-related transcripts in mouse liver. *J Biol Chem* 265:3193-3201.

43. Rosenwasser, T.A., Hogquist, K.A., Nothwehr, S.F., Bradford-Goldberg, S., Olins, P.O., Chaplin, D.D., and Gordon, J.I. (1990): Compartmentalization of mammalian proteins produced in Escherichia-coli. *J Biol Chem* 265:13066-13073.

44. Hagman, J., Rudin, C.M., Haasch, D., Chaplin, D.D., and Storb, U. (1990): A novel enhancer in the immunoglobulin λ locus is duplicated and functionally independent of NFκ B. *Genes Dev* 4:978-992.

45. Kulczycki, A., Webber, J., Soares, H.A., Onken, M.D., Thompson, J.A., Chaplin, D.D., Loh, D.Y., and Tillinghast, J.P. (1990): Genomic organization of mouse Fcγ receptor genes. *Proc Natl Acad Sci U S A* 87:2856-2860.

46. Webb, G.C., and Chaplin, D.D. (1990): Genetic variability at the human tumor necrosis factor α and β loci. In, Molecular and Cellular Biology of Cytokines, eds. Oppenheim, J.J., Powanda, M.C., Kluger, M.J., and Dinarello, C.A., Alan R. Liss, New York, pp 31-35.

47. Webb, G.C., and Chaplin, D.D. (1990): Genetic variability at the human tumor necrosis factor loci. *J Immunol* 145:1278-1285.

48. Wroblewski, J.M., Kaminsky, S.G., Milisauskas, V.K., Pittman, A.M., Chaplin, D.D., Spies, T., and Nakamura, I. (1990): The B144-H-2D^b interval and the location of a murine homologue of the human D6S81E locus. *Immunogenetics* 32:200-204.

49. Howard, A.D., Kostura, M.J., Thornberry, N., Ding, G.J.F., Limjuco, G., Weidner, J., Salley, J.P., Hogquist, K.A., Chaplin, D.D., Mumford, R.A., Schmidt, J.A., and Tocci, M.J. (1991): IL-1-converting enzyme requires aspartic acid residues for processing of the IL-1β precursor at two distinct sites and does not cleave 31-kDa IL-1α. *J Immunol* 147:2964-2969.

50. Bronson, S.K., Pei, J., Taillon-Miller, P., Chorney, M.J., Geraghty, D.E., and Chaplin, D.D. (1991): Isolation and characterization of yeast artificial chromosome clones linking the HLA-B and HLA-C loci. *Proc Natl Acad Sci U S A* 88:1676-1680.

51. Hogquist, K.A., Nett, M.A., Sheehan, K.C.F., Pendleton, K.D., Schreiber, R.D., and Chaplin, D.D. (1991): Generation of monoclonal antibodies to murine IL-1β and demonstration of IL-1 in vivo. *J Immunol* 146:1534-1540.

52. Kozono, H., Bronson, S.K., Taillon-Miller, P., Moorti, M.K., Jamry, I., and Chaplin, D.D. (1991): Molecular linkage of the HLA-DR, HLA-DQ, and HLA-DO genes in yeast artificial chromosomes. *Genomics* 11:577-586.

53. Hogquist, K.A., Unanue, E.R., and Chaplin, D.D. (1991): Release of interleukin-1 from mononuclear phagocytes. *J Immunol* 147:2181-2186.

54. Hogquist, K.A., Nett, M.A., Unanue, E.R., and Chaplin, D.D. (1991): Interleukin-1 is processed and released during apoptosis. *Proc Natl Acad Sci U S A* 88:8485-8489.

55. Parimoo, S., Patanjali, S.R., Shukla, H., Chaplin, D.D., and Weissman, S.M. (1991): cDNA selection: efficient PCR approach for the selection of cDNAs encoded in large chromosomal DNA fragments. *Proc Natl Acad Sci U S A* 88:9623-9627.

56. Chaplin, D.D., and Hogquist, K.A. (1992): Interactions between TNF and Interleukin-1. In, Tumor Necrosis Factors: The Molecules and their Emerging Role in Medicine, ed. Beutler, B., Raven Press, Ltd., New York, chap. 13, pp. 197-220.

57. Domalik, L.J., Chaplin, D.D., Kirkman, M.S., Wu, R.C., Seldin, M.F., Howard, T.A., and Parker, K.L. (1992): Different isozymes of mouse 11β-hydroxylase produce mineralocorticoids and glucocorticoids. *Mol Endocrinol* 5:1853-1861.

58. Chaplin, D.D., and Tillinghast, J.P. (1992): The molecular basis of immune recognition. In, Allergy: Theory and Practice, 2nd edition, eds. Korenblat, P.E., and Wedner, H.J., W. B. Saunders, Philadelphia, pp. 31-60.

59. Bora, N.S., Chaplin, D.D., and Atkinson, J.P. (1992): Restriction fragment length polymorphisms of proteins of the complement system. In, Manual of Clinical Laboratory Immunology, 4th edition, Rose, N.E., de Macario, E.C., Fahey, J.L., Friedman, H., and Penn, G.M., eds., American Society for Microbiology, Washington, D.C., pp. 153-155.

60. Geraghty, D.E., Pei, J., Lipsky, B., Hansen, J., Taillon-Miller, P., Bronson, S.K., and Chaplin, D.D. (1992): Cloning and physical mapping of the HLA class I region spanning the HLA-E to HLA-F interval using yeast artificial chromosomes. *Proc Natl Acad Sci U S A* 89:2669-2673.

61. Nett, M.A., Cerretti, D.P., Berson, D.R., Seavitt, J., Gilbert, D.J., Jenkins, N.A., Copeland, N.G., Black, R.A., and Chaplin, D.D. (1992): Molecular cloning of the murine interleukin-1β converting enzyme cDNA. *J Immunol* 149:3254-3259.

62. Chaplin, D.D., and Brownstein, B.H. (1992): Strategies for screening YAC (yeast artificial chromosome) libraries and for analysis of YAC clones. In Current Protocols in Molecular Biology, eds. Ausubel F., Brent, R., Kingston, R., Moore, D., Seidman, J., Smith, J., and Struhl, K. Current Protocols, New York, units 6.9 and 6.10.

63. Demmer, L.A., and Chaplin, D.D. (1993): Simultaneous transfer of four functional genes from the HLA class II genes region into mammalian cells by fusion with yeast spheroplasts carrying an artificial chromosome. *J Immunol* 150:5371-5378.

64. Hunt, C.R., Gasser, D.L., Chaplin, D.D., Pierce, J.C., and Kozak, C.A. (1993): Chromosomal localization of five murine Hsp70 gene family members: Hsp70-1, Hsp70-2, Hsp70-3, Hsc70t, and Grp78. *Genomics* 16:193-198.

65. Udalova, I.A., Nedospasov, S.A., Webb, G.C., Chaplin, D.D., and Turetskaya, R.L. (1993): Highly informative typing of the human TNF locus using six adjacent polymorphic markers. *Genomics* 16:180-186.

66. Dawson, J., Rordorf-Adam, C., Geiger, T., Towbin, H., Kunz, S., Nguyen, H., Zingel, O., Chaplin, D., and Vosbeck, K. (1993): Interleukin-1 (IL-1) production in a mouse tissue chamber model of inflammation. 1. Development and initial characterization of the model. *Agents Actions* 38:247-254.

67. Dawson, J., Rordorf-Adam, C., Geiger, T., Towbin, H., Kunz, S., Nguyen, H., Zingel, O., Chaplin, D., and Vosbeck, K. (1993): Interleukin-1 (IL-1) production in a mouse tissue chamber model of inflammation. 2. Identification of (tissue) macrophages as the IL-1 producing cells and the effect of anti-inflammatory drugs. *Agents Actions* 38:255-264.

68. Godambe, S.A., Chaplin, D.D., and Bellone, C.J. (1993): Regulation of IL-1 gene expression: differential responsiveness of murine macrophage lines. *Cytokine* 5:327-335.

69. Zhou, Y., and Chaplin, D.D. (1993): Identification in the HLA class I region of a gene expressed late in keratinocyte differentiation. *Proc Natl Acad Sci U S A* 90:9470-9474.

70. Wei, H., Fan, W.-F., Xu, H., Parimoo, S., Shukla, H., Chaplin, D.D., and Weissman, S.M. (1993): Genes in one megabase of the HLA class I region. *Proc Natl Acad Sci U S A* 90:11870-11874.

71. Gasser, D.L., Sternberg, N.L., Pierce, J.C., Goldner-Sauve, A., Feng, J., Haq, A.K., Spies, T., Hunt, C., Buetow, K.H., and Chaplin, D.D. (1994): P1 and cosmid clones define the organization of 280 kb of the mouse *H-2* complex containing the *Cps-1* and *Hsp70* loci. *Immunogenetics* 39:48-55.

72. Godambe, S.A., Chaplin, D.D., Takova, T., and Bellone, C.J. (1994): Upstream NFIL-6-like site located within a DNase I hypersensitivity region mediates LPS-induced transcription of the murine interleukin-1 β gene. *J Immunol* 153:143-152.

73. De Togni, P., Goellner, J., Ruddle, N.H., Streeter, P.R., Fick, A., Mariathasan, S., Smith, S.C., Carlson, R., Shornick, L.P., Strauss-Schoenberger, J., Russell, J.H., Karr, R.W., and Chaplin, D.D. (1994): Abnormal development of peripheral lymphoid organs in mice deficient in lymphotoxin. *Science* 264:703-707.

74. Godambe, S.A., Chaplin, D.D., Takova, T., and Bellone, C.J. (1994): An NFIL-6 sequence near the transcriptional initiation site is necessary for the lipopolysaccharide induction of murine interleukin-1β (IL-1β). *DNA Cell Biol* 13:561-569.

75. Krishnan, B.R., and Chaplin, D.D. (1994): Fluorescent automated sequencing of supercoiled high molecular weight double-stranded DNA. *Biotechniques* 17:854-857.

76. Krishnan, B.R., Jamry, I., Berg, D.E., Berg, C.M., and Chaplin, D.D. (1995): Construction of a genomic DNA 'Feature Map' by sequencing from nested deletions: application to the HLA class I region. *Nucleic Acids Res* 23:117-122.

77. Godambe, S.A., Chaplin, D.D., Takova, T., and Bellone, C.J. (1995): Molecular dissection of the murine IL-1β promoter. *Am J Ther* 2:677-686.

78. Godambe, S.A., Chaplin, D.D., Takova, T., and Bellone, C.J. (1995): A novel cis-acting element required for lipopolysaccharide-induced transcription of the murine IL-1β gene. *Mol Cell Biol* 15:112-119.

79. Chaplin, D.D., and Zhou, Y. (1995): HLA-Linked Skin Disease: Classical HLA genes or novel genes within HLA? *J Invest Dermatol* 104:37S.

80. Krishnan, B.R., Jamry, I., and Chaplin, D.D. (1995): Feature mapping of the HLA Class I region - localization of the POU5F1 and TCF19 genes. *Genomics* 30:53-58.

81. Min, J., Shukla, H., Kozono, H., Bronson, S., Weissman, S., and Chaplin, D.D. (1995): A novel Creb family gene telomeric of HLA-DRA in the HLA complex. *Genomics* 30:149-156.

82. Mariathasan, S., Matsumoto, M., Baranyay, F., Nahm, M., Kanagawa, O., and Chaplin, D.D. (1995): Absence of lymph nodes in lymphotoxin-α (LT-α)-deficient mice is due to abnormal organ development, not defective lymphocyte migration. *J Inflamm* 45:72-78.

83. LaBlanca, F., Krishnan, B.R., Chaplin, D.D., Berg, D.E, and Berg, C.E. (1995): Restriction map of a 35-kb HLA fragment constructed by nested deletion 'drop-out' mapping. *Gene* 164:335-339.

84. Nett-Fiordalisi, M., Thomaselli, K., Russell, J.H., and Chaplin, D.D. (1995): Macrophage apoptosis in the absence of active interleukin-1β converting enzyme. *J Leukoc Biol* 58:717-724.

85. Krishnan, B.R., Young, A., and Chaplin, D.D. (1996): An improved and universal T7-end primer for fluorescent sequencing of nested deletion derivatives obtained using 'Deletion Factory' vectors. *Focus* 18:25-26.

86. Matsumoto, M., Mariathasan, S., Nahm, M.H., Baranyay, F., Peschon, J.J., and Chaplin, D.D. (1996). Role of lymphotoxin and the type I TNF receptor in the formation of germinal centers. *Science* 271:1289-1291.

87. Molina, H., Holers, V.M., Li, B., Fang, Y.-F., Mariathasan, S., Goellner, J., Strauss-Schoenberger, J., Karr, R.W., and Chaplin, D.D. (1996): Markedly impaired humoral immune response in mice deficient in complement receptors 1 and 2. *Proc Natl Acad Sci U S A* 93:3357-3361.

88. Shornick, L.P., De Togni, P., Mariathasan, S., Goellner, J., Strauss-Schoenberger, J., Karr, R.W., Ferguson, T., and Chaplin, D.D. (1996): Mice deficient in IL-1β manifest impaired contact hypersensitivity to trinitrochlorobenzene. *J Exp Med* 183:1427-1436.

89. Matsumoto, M., Lo, S.F., Carruthers, C.J.L., Min, J., Mariathasan, S., Huang, G., Plas, D.R., Martin, S.M., Geha, R.S., Nahm, M.H., and Chaplin, D.D. (1996): Affinity maturation without germinal centers in lymphotoxin-α (LTα) deficient mice. *Nature* 382:462-466.

90. Fu, Y.-X., Huang, G., Matsumoto, M., Molina, H., and Chaplin, D.D. (1997): Independent signals regulate development of primary and secondary follicle structure in spleen and mesenteric lymph node. *Proc Natl Acad Sci U S A* 94:5739-5743.

91. Matsumoto, M., Fu, Y.-X., Molina, H., and Chaplin, D.D. (1997): Lymphotoxin-α-deficient and TNF receptor-I-deficient mice define developmental and functional characteristics of germinal centers. *Immunol Rev* 156:137-144.

92. Fu, Y., Molina, H., Matsumoto, M., Huang, G., Min, J., and Chaplin, D.D. (1997): Lymphotoxin-α (LTα) supports development of splenic follicular structure that is required for IgG responses. *J Exp Med* 185:2111-2120.

93. Matsumoto, M., Fukuda, W., Circolo, A., Goellner, J., Strauss-Schoenberger, J., Wang, X., Fujita, S., Hidvegi, T., Chaplin, D.D., and Colten, H.R. (1997): Abrogation of the alternative complement pathway by targeted deletion of murine factor B. *Proc Natl Acad Sci U S A* 94: 8720-8725.

94. Matsumoto, M., Fu, Y.-X., Molina, H., Huang, G., Kim, J., Thomas, D.A., Nahm, M.H., and Chaplin, D.D. (1997): Distinct roles of lymphotoxin α and the type I tumor necrosis factor (TNF) receptor in the establishment of follicular dendritic cells from non-bone marrow-derived cells. *J Exp Med* 186:1997-2004.

95. Fu, Y., Huang, G., Wang, Y., and Chaplin, D.D. (1998): B lymphocytes induce the formation of follicular dendritic cell clusters in a lymphotoxin-α-dependent fashion. *J Exp Med* 187:1009-1018.

96. Giegel, D.A., and Chaplin, D.D. (1998): Targets in cytokine activation. *Agents Actions Suppl* 49:1-3.

97. Chaplin, D.D., and Fu, Y.-X. (1998): Cytokine regulation of secondary lymphoid organ development. *Curr Opin Immunol* 10:289-297.

98. Randolph, D.A., Carruthers, C.J.L., Szabo, S., Murphy, K.M., and Chaplin, D.D. (1999): Modulation of airway inflammation by passive transfer of allergen-specific Th1 and Th2 cells in a mouse model of asthma. *J Immunol* 162:2375-2383.

99. Fu, Y.-X., and Chaplin, D.D. (1999): Development and maturation of secondary lymphoid tissues. *Annu Rev Immunol* 17:399-433.

100. Iizuka, K., Chaplin, D.D., Wang, Y., Wu, Q., Pegg, L.E., Yokoyama, W.M., and Fu, Y.-X. (1999): Requirement for membrane lymphotoxin in natural killer cell development. *Proc Natl Acad Sci U S A* 96:6336-6340.

101. Grossman, W. J., Verbsky, J. W., Yang, L., Berg, L. J., Fields, L. E., Chaplin, D. D., and Ratner, L. (1999): Dysregulated myelopoiesis in mice lacking Jak3. *Blood* 94:932-939.

102. Randolph, D.A., Stephens, R., Carruthers, C.J.L., and Chaplin, D.D. (1999): Cooperation between Th1 and Th2 cells in a murine model of eosinophilic airway inflammation. *J Clin Invest* 104:1021-1029.

103. Randolph, D.A., Huang, G., Carruthers, C.J.L., Bromley, L.E., and Chaplin, D.D. (1999): The Role of CCR7 in T(H)1 and T(H)2 Cell Localization and Delivery of B Cell Help in Vivo. *Science* 286:2159-2162.

104. Castro, M., Chaplin, D.D., Walter, M.J., and Holtzman, M.J. (2000): Could asthma be worsened by stimulating the T-helper type 1 immune response? *Am J Respir Cell Mol Biol* 22:143-146

105. Fu, Y.-X., Huang, G., Wang, Y., and Chaplin, D.D. (2000): Lymphotoxin-α-dependent spleen microenvironment supports the generation of memory B cells and is required for their subsequent antigen-induced activation. *J Immunol* 164:2508-2514.

106. Wang, Y., Huang, G., Wang, J., Molina, H., Chaplin, D. D., and Fu, Y.-X. (2000): Antigen persistence is required for somatic mutation and affinity maturation of immunoglobulin. *Eur J Immunol* 30:2226-2234.

107. Rennert, P. D., Hochman, P. S., Flavell, R. A., Chaplin, D. D., Jayaraman, S., Browning, J. L., and Fu, Y.-X. (2001): Essential role of lymph nodes in contact hypersensitivity revealed in lymphotoxin-α-deficient mice. *J Exp Med* 193:1227-1238.

108. Hussain, I., Randolph, D., Brody, S. L., Song, S. K., Hsu, A., Kahn, A. M., Chaplin, D. D., and Hamilos, D. L. (2001): Induction, distribution and modulation of upper airway allergic inflammation in mice. *Clin Exp Allergy* 31:1048-1059.

109. Mandik-Nayak, L., Huang, G., Sheehan, K.C.F., Erikson, J., and Chaplin, D.D. (2001): Signaling through TNF receptor p55 in TNF-α-deficient mice alters the CXCL13/CCL19/CCL21 ratio in the spleen and induces maturation and migration of anergic B cells into the B cell follicle. *J Immunol* 167:1920-1928.

110. Grayson, M. H., Chaplin, D. D., Karl, I. E., and Hotchkiss, R. S. (2001): Confocal fluorescent intravital microscopy of the murine spleen. *J Immunol Methods* 256:55-63.

111. Shornick, L.P., Bisarya, A.K., and Chaplin, D.D. (2001): IL-1β is essential for Langerhans cell activation and antigen delivery to lymph nodes during contact sensitization: evidence for a dermal source of IL-1β. *Cellular Immunol* 211:105-112.

112. Dube, P.H., Revell, P.A., Chaplin, D.D., Lorenz, R.G., and Miller, V.L. (2001): A role for IL-1α in inducing pathologic inflammation during bacterial infection. *Proc. Natl. Acad. Sci. USA* 98:10880-10885.

113. Mason, J.L., Suzuki, K., Chaplin, D.D., and Matsushima, G.K. (2001): Interleukin-1β promotes repair of the CNS. *J Neurosci 21*:7046-7052.

114. Byersdorfer, C.A., and Chaplin, D.D. (2001); Visualization of early APCT/T cell interactions in the mouse lung following intranasal challenge. *J Immunol* 167:6756-6764.

115. Verbsky, J.W., Randolph, D.A., Shornick, L.P., and Chaplin, D.D. (2002): Nonhematopoietic expression of Janus Kinase 3 is required for efficient recruitment of Th2 lymphocytes and eosinophils in OVA-induced airway inflammation. *J Immunol* 168:2475-2482.

116. Stephens, R., Eisenbarth, S.C., Chaplin, D.D. (2002): T helper type 1 cells in asthma: friend or foe? *Curr Opin Allergy Clin Immunol* 2:31-37.

117. Chaplin, D.D. (2002): Regulation of spleen white pulp structure and function by lymphotoxin. *Adv Exp Med Biol* 512:49-56.

118. Chaplin, D.D. (2002): Cell cooperation in the development of eosinophil-predominant inflammation in airways. *Immunol Res* 26:55-62.

119. Stephens, R., Randolph, D.A., Huang, G., Holtzman, M.J., Chaplin, D.D. (2002): Antigen-non-specific recruitment of Th2 cells to the lung as a mechanism for viral infection-induced allergic asthma. *J Immunol* 169:5458-5467.

120. Stephens, R., Chaplin, D.D. (2002): IgE cross-linking or lipopolysaccharide treatment induces recruitment of Th2 cells to the lung in the absence of specific antigen. *J Immunol* 169:5468-5476.

121. Grayson, M.H., Chaplin, D.D. (2002): Localization of T and B lymphocytes to the white pulp of the spleen is independent of L-, E-, and P-selectin. *TheScientificWorldJOURNAL* 3:484-496.

122. Chaplin, D.D. (2003): Overview of the Immune Response. *J Allergy Clin Immunol* 111:S442-459.

123. Grayson, M.H., Hotchkiss, R.S., Karl, I.E., Holtzman, M.J., Chaplin, D.D. (2003): Intravital microscopy comparing T lymphocyte trafficking to the spleen and the mesenteric lymph node. *Am J Physiol Heart Circ Physiol* 284:H2213-H2226.

124. Lorenz, R.G., Chaplin, D.D., McDonald, K.G., McDonough, J.S., and Newberry, R.D. (2003): Isolated lymphoid follicle formation is inducible and dependent upon lymphotoxin-sufficient B lymphocytes, lymphotoxin β receptor, and TNF receptor I function. *J Immunol* 170:5475-5482.

125. Chaplin, D.D. (2003): Lymphoid Tissues and Organs. In, *Fundamental Immunology, 5th Ed.,* Paul, W.E, ed. Lippincott Williams & Wilkins, Philadelphia.

Invited Lectures:

Jan. 26, 1984	The Royal Society of London, Biochemistry and Genetics of Complement: Cloning and expression of murine C4 and Slp.
Dec. 12, 1988	Univ. of Missouri, Dept. of Microbiology: Molecular immunology of Interleukin-1.
Dec. 17, 1991	Univ. of Texas Medical Branch at Galveston: Interleukin-1, a secreted cytokine?
Nov. 7, 1994	National Workshop on Alopecia Areata: HLA-linked skin disease: classical HLA genes or novel genes within HLA?
Jan. 31, 1995	Ohio State Univ.: Molecular Analysis of the HLA Complex.
Aug. 25, 1995	BASF BioResearch Corp: Gene Targeting to Define the Role of IL-1β *in vivo.*
Feb. 15, 1996	Barnes-Jewish Medical Grand Rounds: Gene Targeting to Define the *in Vivo* Functions of Cytokines
May 10, 1996	6th International Congress, TNF and Related Molecules, Rhodes, Greece: Lymphotoxin-α-Deficient and TNF-Receptor I-Deficient Mice Define Developmental and Functional Characteristics of Germinal Centers.
May 21, 1996	St. Louis Jewish Hospital Grand Rounds: Gene Targeting to Define the *in Vivo* Functions of Cytokines
Oct. 28, 1996	Chairman, Inflammation Research Association Conference Session: Targets and Cytokine Action
Dec. 16, 1996	University of Washington Immunology Program: Role of Lymphotoxin in Peripheral Lymphoid Tissue Development
Dec. 17, 1996	Immunex Corp.: Essential Role of IL-1β in Contact Hypersensitivity Responses
Feb. 13, 1997	Biogen Corp.: Role of Lymphotoxin in Peripheral Lymphoid Tissue Development
Mar. 20, 1997	New York University School of Medicine/Skirball Institute: Essential Role of Lymphotoxin in Peripheral Lymphoid Tissue Development
Apr. 11, 1997	University of Utah, Developmental Biology Program: Cytokine Signals for Lymphoid Tissue Development
May 21, 1997	Pfizer Corp.: Role of Lymphotoxin in Peripheral Lymphoid Tissue Development
May 22, 1997	Inflammation Research Association: Induction of IL-1 During Apoptosis

June 24, 1997	FASEB Conference on Autoimmunity: Cytokine Signals for Lymphoid issue Development
July 1, 1997	Gordon Conference: Lymphotoxin, a Primary Determinant of Lymphoid Tissue Structure
Oct.8, 1997	National Jewish Center for Immunology and Respiratory Diseases: Lymphotoxin, a Primary Determinant of Lymphoid Tissue Structure
Dec. 3, 1997	Duke University, Department of Immunology: Role of Lymphotoxin in Peripheral Lymphoid Tissue Development
Jan. 27, 1998	37th Midwinter Immunology Conference, Asilomar: Lymphotoxin-Dependent Signals Controlling Peripheral Lymphoid Tissue Development
Feb. 19, 1998	University of North Carolina, Department of Microbiology: Lymphotoxin-Dependent Signals Controlling Peripheral Lymphoid Tissue Development
Mar. 2, 1998	University of Rochester, Department of Pediatrics: Lymphotoxin-Dependent Signals Controlling Peripheral Lymphoid Tissue Development
May 20, 1998	7th International TNF Congress, Hyannis: Lymphotoxin-Dependent Signals Controlling Peripheral Lymphoid Tissue Development
June 23, 1998	FASEB Conference on Lymphocytes and Antibodies: TNF/LT Family Members as Signals for Lymphoid Tissue Development
June 26, 1998	International Union of Immunological Societies, Symposium on Primary Immunodeficiency Diseases: Cytokine Signals for the Development of Primary B Cell Follicle Structure
Sept. 9, 1998	St. Jude Children's Research Hospital, Department of Immunology: Lymphotoxin-Dependent Signals Controlling Peripheral Lymphoid Tissue Development
Oct. 27, 1998	International Cytokine Society, Jerusalem: Lymphotoxin-Dependent Signals Regulating Primary B Cell Follicle Structure and Function
Dec. 7, 1998	Washington University Center for Immunology Seminar: Signals Controlling Normal Lymphoid Tissue Structure and Function
Dec. 9, 1998	Wistar Institute: Lymphotoxin, a Major Determinant for Normal Secondary Lymphoid Tissue Development and Function
Jan. 26, 1999	Vanderbilt University, Department of Microbiology and Immunology: Signals Controlling Normal Lymphoid Tissue Structure and Function

Feb. 11, 1999	Keystone Conference: B Lymphocyte Biology and Disease TNF Family Members in Formation of Primary Lymphoid Follicles
Feb. 27, 1999	American Academy of Allergy, Asthma and Immunology, 55th Annual Meeting: Synergy of Th1 and Th2 Cells in Experimental Eosinophilic Airway Inflammation
Mar. 15, 1999	University of Toronto, Immunology Department Seminar Series: Cellular and Molecular Determinants of Peripheral Lymphoid Tissue Structure and Function
May 8, 1999	Nikolas Symposium, Athens, Greece: Cytokines and Lymphoid Tissue Development
Sept. 25, 1999	National Residency Education Program, American Association of Allergy, Asthma, and Immunology, St. Louis, MO: Allergy-Immunology: from Bench to Bedside.
Oct. 22, 1999	Allergy Abroad, Paris, France: Cooperation Between T Helper Cells in Allergic Airway Inflammation
Oct. 26, 1999	Allergy Abroad, Lyon, France: Control of Lymphocyte Movement and Function by Chemokines
Oct. 29, 1999	Allergy Abroad, Montpellier, France Organization and Function of Secondary Lymphoid Tissues
Nov. 9, 1999	Stanford University, Program in Immunology Seminar: Regulation of Lymphoid Tissue Structure and Function
Nov. 30, 1999	Kyoto University, Department of Molecular Genetics: Regulation of Lymphoid Tissue Structure and Function
Dec. 2, 1999	Kyoto, Japan, 29th Annual Meeting of the Japanese Society for Immunology, Symposium on Lymphocyte Development in Germinal Centers: Targeting within Secondary Lymphoid Tissues and Control of Antibody Responses
Apr. 5, 2000	University of Alabama at Birmingham, Department of Microbiology Regulation of Lymphoid Tissue Structure and Function
Apr. 17, 2000	NIAID/NCI Symposium: Cells of the Marginal Zone – Origins, Function and Neoplasia, Bethesda, MD: Regulation of secondary lymphoid tissue follicle structure and function by lymphotoxin
May 13, 2000	AAI Annual Meeting, Seattle, WA. Major Symposium Co-Chair: Molecular Mechanisms of Lymphoid Organogenesis. Regulation of secondary lymphoid tissue follicle structure and function by lymphotoxin
Aug. 19, 2000	Clinical Allergy for the Practicing Physician, St. Louis, MO. DNA Vaccines

Sept. 9, 2000	1st International Workshop on Nucleotides and Their Receptors in the Immune System, Ferrara, Italy Is apoptosis required for IL-1 action *in vivo*?
Oct. 3, 2000	Howard Hughes Medical Institute: Infection and Immunity Molecular Determinants of Spleen Follicle Structure and Function
Oct. 25, 2000	University of Iowa, Department of Microbiology Regulation of secondary lymphoid tissue follicle structure and function by lymphotoxin
Jan. 17, 2001	Albert Einstein College of Medicine, Division of Biological Sciences Seminar Series Molecular Determinants of Spleen Follicle Structure and Function
Mar. 12, 2001	Washington University Center for Immunology Seminar: Regulation of Secondary Lymphoid Tissue Structure and Function by Lymphotoxin and TNF
Mar. 18, 2001	57th Annual Meeting of the American Academy of Allergy, Asthma and Immunology, New Orleans, LA: Grand Seminar. Regulation of Secondary Lymphoid Tissue Structure and Function by Lymphotoxin
Apr. 19, 2001	New York University Immunology Program Seminar: Mechanisms Regulating Th2-dependent Inflammation in Peripheral Tissues
May 23, 2001	Mucosal Immunology at the 21st Century, Perdido Beach, AL: Plasticity of Secondary Lymphoid Tissue Structures
June 7, 2001	NIH/NIAID Asthma Center Directors Meeting, Bethesda, MD: Regulation of T Helper Cell Recruitment to Peripheral Tissues
July 23, 2001	11th International Congress of Immunology, Stockholm, Sweden: Symposium on Antigen Processing and Presentation at Mucosal Surfaces. Control of Lymphoid Tissue Structure and Function by LT and TNF
Nov. 6, 2001	EU and NIH Conference, Siena, Italy: Potential Impact of New Technologies on Vaccination in Early Life. Signals for Development of Secondary Lymphoid Organs
Dec. 5, 2001	British Society for Immunology Annual Congress, Harrogate, UK: Plenary Speaker. Recruitment of Th2 Cells to Peripheral Sites *in vivo*
Jan. 22, 2002	Department of Microbiology, University of Alabama at Birmingham: Recruitment of Th2 Cells to Peripheral Sites *in vivo*
Feb. 8, 2002	9th International Conference on Lymphocyte Traffic and Homeostasis, Newport Beach, CA: Structural Elements Regulating Lymphocyte Trafficking to and in the Spleen
Mar. 2, 2002	58th Annual Meeting of the American Academy of Allergy, Asthma and Immunology,

New York, NY: Role of Inflammation in Recruitment of Th2 Lymphocytes to the Lung

June 9, 2002 FASEB Conference, Anatomy of the Immune Response *in vivo*, Snowmass, CO: Lymphocyte Trafficking Patterns in the Spleen

Exhibit A.

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Beutler, *et al.*

Serial No.: 09/396,985

Filed: September 15, 1999

For: LPS-RESPONSE GENE COMPOSITIONS AND METHODS

Group Art Unit: 1646

Examiner: Basi, N.

Atty. Dkt. No.: UTSD:602

CERTIFICATE OF MAILING
37 C.F.R. § 1.8

I hereby certify that this correspondence is being deposited with the U.S. Postal Service as First Class Mail in an envelope addressed to: Commissioner for Patents, Washington, D.C. 20231, on the date below:

October 23, 2003
Date

Mark B. Wilson

AMENDMENT UNDER 37 C.F.R. 1.116
SUBMITTED WITH APPEAL BRIEF

MAIL STOP APPEAL BRIEF - PATENTS
Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

Commissioner:

This amendment is filed solely to resolve a minor editing issue, and is filed in conjunction with the currently filed Appeal Brief. Since no new matter or issues are introduced or raised by the amendment, entry of the amendment is respectfully requested. No fees are believed due in connection with this amendment; however, should any fees be due, the Commissioner is authorized to debit Fulbright & Jaworski Deposit Account No. 50-1212/UTSD:602/MBW. Amendments to the claims begin on page 2 of this paper. Remarks begin on page 6 of this paper.

AMENDMENT

Listing of Claims:

The following listing of claims replaces all previous listings or versions thereof:

1-37. (Canceled)

38. (Previously Presented) A method of screening for modulators of a lipopolysaccharide mediated response comprising the steps of:

a) obtaining a cell expressing a TLR-4 polypeptide;

b) measuring a lipopolysaccharide mediated response mediated by the TLR-4 polypeptide;

c) contacting the TLR-4 polypeptide with a putative modulator;

d) assaying for a change in the lipopolysaccharide mediated response; and

e) comparing the lipopolysaccharide mediated responses mediated by the TLR-4 polypeptide obtained in steps b) and d) above

wherein a difference in the lipopolysaccharide mediated responses indicates that the putative modulator is a modulator of a lipopolysaccharide mediated response.

39. (Original) The method of claim 38, wherein the TLR-4 polypeptide has the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:98 or SEQ ID NO:99.

40. (Previously Presented) The method of claim 39, wherein the lipopolysaccharide mediated response mediated by the TLR-4 polypeptide is determined by determining the ability of the TLR-4 polypeptide to stimulate transcription of a reporter gene, the reporter gene operatively positioned under control of a nucleic acid segment comprising a promoter from a TLR-4 gene.

41-51. (Canceled)

52. (Previously Presented) The method of claim 38, wherein said putative modulator is effective in altering the mediation of the lipopolysaccharide mediated response by TLR-4.

53. (Previously Presented) The method of claim 52, wherein said putative modulator is an agonist.

54. (Previously Presented) The method of claim 52, wherein said putative modulator is an antagonist.

55. (Previously Presented) The method of claim 52, wherein said putative modulator affects the transcription of TLR-4.

56. (Previously Presented) The method of claim 52, wherein said putative modulator affects the translation of TLR-4.

57. (Previously Presented) The method of claim 38, wherein the TLR-4 polypeptide has the amino acid sequence of SEQ ID NO:2.

58. (Previously Presented) The method of claim 38, wherein the TLR-4 polypeptide has the amino acid sequence of SEQ ID NO:4.

59. (Previously Presented) The method of claim 38, wherein the TLR-4 polypeptide has the amino acid sequence of SEQ ID NO:6.

60. (Previously Presented) The method of claim 38, wherein the TLR-4 polypeptide has the amino acid sequence of SEQ ID NO:98.

61. (Previously Presented) The method of claim 38, wherein the TLR-4 polypeptide has the amino acid sequence of SEQ ID NO:99.

62. (Canceled)

63. (Previously Presented) The method of claim 38, wherein said putative modulator inhibits TLR-4 directed signaling of TNF secretion.

64. (Previously Presented) The method of claim 38, wherein said putative modulator stimulates TLR-4 directed signaling of TNF secretion.

65. (Previously Presented) The method of claim 38, wherein said putative modulator to be screened is obtained from a library of synthetic chemicals.

66. (Previously Presented) The method of claim 38, wherein said putative modulator to be screened is obtained from a natural source.

67. (Currently Amended) The method of claim ~~6566~~, wherein said natural source is selected from the group consisting of animals, bacteria, fungi, plant sources and living marine samples.

68. (Previously Presented) The method of claim 38, wherein said putative modulator to be screened is a protein or peptide.

69. (Canceled)

70. (Previously Presented) The method of claim 38, wherein said putative modulator to be screened is a nucleic acid molecule.

71. (Previously Presented) The method of claim 38, wherein said putative modulator to be screened is a stimulator of an immune response.

72. (Previously Presented) The method of claim 71, wherein said stimulator of an immune response is a cytokine.

73. (Previously Presented) The method of claim 71, wherein said stimulator of an immune response is an interferon.

74. (Previously Presented) The method of claim 38, wherein said TLR-4 polypeptide is encoded by a nucleic acid sequence selected from the group comprising SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:46, SEQ ID NO:47 and SEQ ID NO:48.

75. (Previously Presented) The method of claim 38, wherein said putative modulator to be screened is an IL-1 receptor antagonist.

76-99. (Canceled)

100. (Previously Presented) The method of claim 38, wherein the TLR-4 polypeptide has the amino acid sequence selected from the group comprising SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:98 and SEQ ID NO:99.

101. (Previously Presented) The method of claim 38, wherein said putative modulator to be screened is a small molecule.

102. (Previously Presented) The method of claim 101, wherein said small molecule inhibits TLR-4 mediation of the lipopolysaccharide mediated response.

103. (Previously Presented) The method of claim 101, wherein said small molecule inhibits the lipopolysaccharide mediated response.

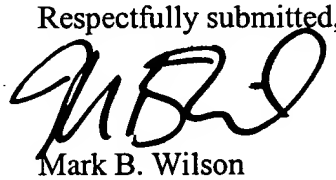
REMARKS

Claims 38-40, 52-61, 63-68, 70-75, and 100-103 were pending at the time of this amendment. Claim 67 has been amended to correct an incorrect dependency. Therefore, claims 38-40, 52-61, 63-68, 70-75, and 100-103 are pending.

Applicants respectfully submit that this amendment places to claims in even better condition for allowance or appeal by resolving a minor typographical error. Therefore, entry of this amendment is respectuflly requested.

Should the examiner have any questions or comments regarding this amendment, a telephone call to the undersigned Applicants' representative is respectfully requested.

Respectfully submitted,



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Date: October 23, 2003

Exhibit B

Exhibit B

Scientific Literature Referring to TLR-4, TLR4, tlr4, or toll-like receptor 4.

1. Poltorak,A., X.He, I.Smirnova, M.-Y.Liu, C.Van Huffel, X.Du, D.Birdwell, E.Alejos, M.Silva, C.Galanos, M.A.Freudenberg, P.Ricciardi-Castagnoli, B.Layton, and B.Beutler. 1998. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in *Tlr4* gene. *Science* 282:2085-2088.

2. Du, X., Poltorak, A., Silva, M., and Beutler, B. Analysis of Tlr4-mediated LPS signal transduction in macrophages by mutational modification of the receptor. Blood Cells Molecules & Diseases 25(21), 328-338. 11-8-1999.

3. Frantz, S., Kobzik, L., Kim, Y. D., Fukazawa, R., Medzhitov, R., Lee, R. T., and Kelly, R. A. Toll4 (TLR4) expression in cardiac myocytes in normal and failing myocardium. J.Clin.Invest. 104(3), 271-280. 1999.

4. Hoshino,K., O.Takeuchi, T.Kawai, H.Sanjo, T.Ogawa, Y.Takeda, Takeda, and S.Akira. 1999. Cutting edge: Toll-like receptor 4 (TLR4)-deficient mice are hyporesponsive to lipopolysaccharide: Evidence for TLR4 as the Lps gene product. *Journal of Immunology* 162:3749-3752.

5. Qureshi,S.T., L.Larivière, G.Leveque, S.Clermont, K.J.Moore, P.Gros, and D.Malo. 1999. Endotoxin-tolerant mice have mutations in toll-like receptor 4 (Tlr4) [CORRECTION]. *J.Exp.Med.* 189:1519-1520.

6. Qureshi,S.T., L.Lariviere, G.Leveque, S.Clermont, K.J.Moore, P.Gros, D.Malo, Lipopolysaccharide, Inflammation, c.Positional, Salmonella, and Mice. 1999. Endotoxin-tolerant mice have mutations in toll-like receptor 4 (Tlr4). *J.Exp.Med.* 189:615-625.

7. Shimazu, R., Akashi, S., Ogata, H., Nagai, Y, Fukudome, K., Miyake, K., and Kimoto, M. MD-2, a molecule that confers lipopolysaccharide responsiveness on Toll-like receptor 4. Journal of Experimental Medicine 189(11), 1777-1782. 6-7-1999.

8.Takeuchi, O., Hoshino, K., Kawai, T., Sanjo, H., Takada, H., Ogawa, T., Takeda, K., and Akira, S. Differential roles of TLR2 and TLR4 in recognition of Gram-negative and Gram-positive bacterial cell wall components. Immunity 11, 443-451. 1999.

9.. Akashi,S., R.Shimazu, H.Ogata, Y.Nagai, K.Takeda, M.Kimoto, and K.Miyake. 2000. Cutting edge: cell surface expression and lipopolysaccharide signaling via the toll-like receptor 4-MD-2 complex on mouse peritoneal macrophages [In Process Citation]. *J.Immunol.2000.Apr.1.;164.(7.):3471.-5.* 164:3471-3475.

10. Arbour, N. C., Lorenz, E., Schutte, B. C., Zabner, J., Kline, J. N., Jones, M., Frees, K., Watt, J. I., and Schwartz, D. A. *TLR4* mutations are associated with endotoxin hyporesponsiveness in humans. Nature Genetics 25, 187-192. 2000.

11. Beutler, B. Endotoxin, Toll-like receptor 4, and the afferent limb of innate immunity. Curr.Opin.Microbiol. 3(1), 23-28. 2000.

12. Beutler, B. Tlr4: central component of the sole mammalian LPS sensor. Curr.Opin.Immunol. 12(1), 20-26. 2000.

13. Beutler, B., Smirnova, I., and Poltorak, A. Tlr4: the sole gateway to endotoxin response. Proceedings of the 1st Regensburg Immunology Congress . 2000.

14. Hou,L., H.Sasaki, and P.Stashenko. 2000. Toll-Like Receptor 4-Deficient Mice Have Reduced Bone Destruction following Mixed Anaerobic Infection. *Infect.Immun.* 68:4681-4687.

15. Jiang,Q., S.Akashi, K.Miyake, and H.R.Petty. 2000. Lipopolysaccharide induces physical proximity between CD14 and toll- like receptor 4 (TLR4) prior to nuclear translocation of NF-kappaB [In Process Citation]. *J.Immunol.* 165:3541-3544.

16. Kawasaki,K., S.Akashi, R.Shimazu, T.Yoshida, K.Miyake, and M.Nishijima. 2000. Mouse toll-like receptor 4.MD-2 complex mediates lipopolysaccharide-mimetic signal transduction by Taxol. *J.Biol.Chem.2000.Jan.28.;275.(4.):2251.-4.* 275:2251-2254.

17. Kleeberger,S.R., S.Reddy, L.Y.Zhang, and A.E.Jedlicka. 2000. Genetic susceptibility to ozone-induced lung hyperpermeability. Role of toll-like receptor 4. *Am.J.Respir.Cell Mol.Biol.* 22:620-627.

18. Kurt-Jones, E. A., Popova, L., Kwinn, L., Haynes, L. M., Jones, L. P., Tripp, R. A., Walsh, E. E., Freeman, M. W., Golenbock, D. T., Anderson, L. J., and Finberg, R. W. Pattern recognition receptors TLR4 and CD14 mediate response to respiratory syncytial virus. Nature Immunology 5, 398-401. 2000.

19. Lien,E., T.K.Means, H.Heine, A.Yoshimura, S.Kusumoto, K.Fukase, M.J.Fenton, M.Oikawa, N.Qureshi, B.Monks, R.W.Finberg, R.R.Ingalls, and D.T.Golenbock. 2000. Toll-like receptor 4 imparts ligand-specific recognition of bacterial lipopolysaccharide. *J.Clin.Invest.* 105:497-504.

20. Lien,E., J.C.Chow, L.D.Hawkins, P.D.McGuinness, K.Miyake, T.Espevik, F.Gusovsky, and D.T.Golenbock. 2000. A novel synthetic acyclic lipid A-like agonist activates cells via the lipopolysaccharide/Toll-like receptor 4 signaling pathway. *J.Biol.Chem.*

21. Matsuguchi,T., T.Musikacharoen, T.Ogawa, and Y.Yoshikai. 2000. Gene Expressions of Toll-Like Receptor 2, But Not Toll-Like Receptor 4, Is Induced by LPS and Inflammatory Cytokines in Mouse Macrophages. *J.Immunol.* 165:5767-5772.

22. Matsumura,T., A.Ito, T.Takii, H.Hayashi, and K.Onozaki. 2000. Endotoxin and cytokine regulation of toll-like receptor (TLR) 2 and TLR4 gene expression in murine liver and hepatocytes [In Process Citation]. *J.Interferon Cytokine Res.* 20:915-921.

23. Nattermann,J., X.Du, Y.Wei, D.Shevchenko, and B.Beutler. 2000. Endotoxin-mimetic effect of antibodies against Toll-like receptor 4. *J.Endotoxin Res.* 6:257-264.

24. Nomura,F., S.Akashi, Y.Sakao, S.Sato, T.Kawai, M.Matsumoto, K.Nakanishi, M.Kimoto, K.Miyake, K.Takeda, and S.Akira. 2000. Cutting edge: endotoxin

tolerance in mouse peritoneal macrophages correlates with down-regulation of surface toll-like receptor 4 expression [In Process Citation]. *J.Immunol.2000.Apr.1.;164.(7.):3476.-9.* 164:3476-3479.

25. Poltorak, A., Smirnova, I., Clisch, R., and Beutler, B. Limits of a deletion spanning *Tlr4* in C57BL/10ScCr mice. J.Endotoxin Res. 6(1), 51-56. 2000.

26. Poltorak, A., Ricciardi-Castagnoli, P., Citterio, A., and Beutler, B. Physical contact between LPS and Tlr4 revealed by genetic complementation. Proceedings of the National Academy of Sciences of the United States of America 97(5), 2163-2167. 2000.

27. Rehli, M., Poltorak, A., Schwarzfischer, L., Krause, S. W, Andreesen, R., and Beutler, B. PU.1 and interferon consensus sequence binding protein (ICSBP) regulate the myeloid expression of the human Toll-like receptor 4 gene. Journal of Biological Chemistry 275(13), 9773-9781. 2000.

28. Rhee,S.H. and D.Hwang. 2000. Murine TOLL-like Receptor 4 Confers Lipopolysaccharide Responsiveness as Determined by Activation of NFkappa B and Expression of the Inducible Cyclooxygenase. *J.Biol.Chem.* 275:34035-34040.

29. Sato,S., F.Nomura, T.Kawai, O.Takeuchi, P.F.Muhlradt, K.Takeda, and S.Akira. 2000. Synergy and cross-tolerance between toll-like receptor (TLR) 2- and TLR4-mediated signaling pathways [In Process Citation]. *J.Immunol.* 165:7096-7101.

30. Smirnova, I., Poltorak, A., Chan, E. K. L., McBride, C., and Beutler, B. Phylogenetic variation and polymorphism at the Toll-like receptor 4 locus (TLR4). Genome Biology 1(1), 1-10. 2000.

31. Tapping,R.I., S.Akashi, K.Miyake, P.J.Godowski, and P.S.Tobias. 2000. Toll-Like Receptor 4, But Not Toll-Like Receptor 2, Is a Signaling Receptor for Escherichia and Salmonella Lipopolysaccharides. *J.Immunol.* 165:5780-5787.

32. Wang,P.L., Y.Azuma, M.Shinohara, and K.Ohura. 2000. Toll-like Receptor 4-Mediated Signal Pathway Induced by Porphyromonas gingivalis Lipopolysaccharide in Human Gingival Fibroblasts. *Biochem.Biophys.Res.Commun.* 273:1161-1167.

33. Yang,H., D.W.Young, F.Gusovsky, and J.C.Chow. 2000. Cellular Events Mediated by Lipopolysaccharide-stimulated Toll-like Receptor 4. MD-2 IS REQUIRED FOR ACTIVATION OF MITOGEN-ACTIVATED PROTEIN KINASES AND Elk-1. *J.Biol.Chem.* 275:20861-20866.

34. Beutler, B. and Poltorak, A. The sole gateway to endotoxin response: how *Lps* was identified as *Tlr4*, and its role in innate immunity. Drug Metab.Dispos. 29, 474-478. 1-1-2001.

35. Beutler,B., X.Du, and A.Poltorak. 2001. Identification of Toll-like receptor 4 (TLR4) as the sole conduit for LPS signal transduction: genetic and evolutionary studies. *J.Endotoxin Res.* In press.

36. Bihl,F., L.Lariviere, S.T.Qureshi, L.Flaherty, and D.Malo. 2001. LPS-hyporesponsiveness of mnd mice is associated with a mutation in Toll-like receptor 4. *Genes Immun.* 2:56-59.

37. Byrd-Leifer,C.A., E.F.Block, K.Takeda, S.Akira, and A.Ding. 2001. The role of MyD88 and TLR4 in the LPS-mimetic activity of Taxol. *Eur.J.Immunol.* 31:2448-2457.

38. da Silva,C.J., K.Soldau, U.Christen, P.S.Tobias, and R.J.Ulevitch. 2001. Lipopolysaccharide is in Close Proximity to Each of the Proteins in Its Membrane Receptor Complex: Transfer from CD14 to TLR4 and MD-2. *J.Biol.Chem.*

39. Pridmore,A.C., D.H.Wyllie, F.Abdillahi, L.Steeghs, L.P.van Der, S.K.Dower, and R.C.Read. 2001. A Lipopolysaccharide-Deficient Mutant of Neisseria meningitidis Elicits Attenuated Cytokine Release by Human Macrophages and Signals via Toll-like Receptor (TLR) 2 but Not via TLR4/MD2. *J.Infect.Dis.* 183:89-96.

40. Read,R.C., J.Pullin, S.Gregory, R.Borrow, E.B.Kaczmarski, F.S.Di Giovine, S.K.Dower, C.Cannings, and A.G.Wilson. 2001. A Functional Polymorphism of Toll-like Receptor 4 Is Not Associated with Likelihood or Severity of Meningococcal Disease. *J.Infect.Dis.* 184:640-642.

41. Sasu,S., D.LaVerda, N.Qureshi, D.T.Golenbock, and D.Beasley. 2001. Chlamydia pneumoniae and chlamydial heat shock protein 60 stimulate proliferation of human vascular smooth muscle cells via toll-like receptor 4 and p44/p42 mitogen-activated protein kinase activation. *Circ.Res.* 89:244-250.

42. Smiley,S.T., J.A.King, and W.W.Hancock. 2001. Fibrinogen stimulates macrophage chemokine secretion through toll-like receptor 4. *J.Immunol.* 167:2887-2894.

43. Smirnova,I., M.Hamblin, C.McBride, B.Beutler, and A.Di Rienzo. 2001. Excess of rare amino acid polymorphisms in the Toll-like receptor 4 in humans. *Genetics* 158:1657-1664.

Exhibit C

CERTIFICATE OF MAILING
37 C.F.R §1.8

I hereby certify that this correspondence is being deposited with the U.S. Postal Service as First Class Mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231, on the date below:

23 October 2002
Date

Thomas M. Boyce

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Beutler, et al.

Serial No.: 09/396,985

Filed: September 15, 1999

For: LPS-RESPONSE GENE COMPOSITIONS AND METHODS

Group Art Unit: 1646

Examiner: Basi, N.

Atty. Dkt. No.: UTSD:602/TMB

DECLARATION OF DAVID D. CHAPLIN, M.D., Ph.D.

I, David D. Chaplin, hereby declare as follows:

1. I am a U.S. citizen residing at 406 Wildwood Lane, Indian Springs, Alabama 34124. I am the Charles H. McCauley Professor and Chairman of the Department of Microbiology at the University of Alabama at Birmingham. I have extensive experience in the study of cellular responses to endotoxins. References containing examples of my work are included in my *Curriculum Vitae*. A copy of my *Curriculum Vitae* is attached as Exhibit 1.

2. I understand that the present invention relates to methods for screening for modulators of TLR-4 mediated responses to lipopolysaccharide (LPS) mediated responses. The methods involve the use of a TLR-4 polypeptide and the measurement of LPS mediated responses, themselves mediated by TLR-4, in the presence and absence of a putative modulatory compound.

3. I understand that the patent examiner in charge of assessing the patentability of the above-referenced application has rejected the claims of that application on a variety of grounds. I have reviewed the Office Action dated April 23, 2002, the specification of the application and the pending claims. In light of these documents, and my knowledge of the field of endotoxins and cellular biology, I make the following statements.

4. I understand that the examiner has asserted that skilled cellular biologists would not clearly understand the scope of the claims since they recite measurement of a "lipopolysaccharide mediated response." The examiner has asserted that a "lipopolysaccharide mediated response" is not clearly defined in the specification or in the knowledge of the field of endotoxin biology. I do not find this to be the case.

5. The specification clearly sets forth the actors and elements of lipopolysaccahride mediated responses that are mediated by TLR-4. For example, see pages 87-88, which refer to TNF production and splenocyte proliferation assays, commonly employed assays for LPS response.

6. Furthermore, a skilled researcher in endotoxin biology, relying upon the generally available knowledge in the field, would understand that in the context of the application the "lipopolysaccharide pathway" is the cellular response mounted by the action of lipopolysaccharide endotoxins mediated by TLR-4. As disclosed in the specification and as known to the researcher in the field, one may measure such responses through a variety of means, each identifying and measuring responses at a particular point in the signaling pathway.

7. The examiner has rejected several claims because the examiner believes that the name "TLR-4" is not definitive of particular proteins. The examiner states that insufficient structural

and functional properties have been presented in the specification to allow the proper identification of a TLR-4 protein. I do not find this to be the case.

8. Contrary to the examiner's position, my reading of the application provides me with at least sufficient structural and functional properties by which to identify a protein as TLR-4 or its homolog. The particular name associated with TLR-4 and its homologs is not determinative of their identity. Rather, its is their structure, primarily the similarity of the amino acid sequences among members of the TLR-4 family, and their function, primarily their role in mediating responses to endotoxins, that identifies TLR-4 polypeptides.

9. First, the family of TLR-4 receptors share high sequence similarities in specific domains, identifiable by their shared sequence motifs, as provided by the application. See, for example, pages 110-122.

10. Second, the domains of TLR-4 have specific functions, as described in the application. Primarily, TLR-4 polypeptides act to signal the presence of LPS. TLR-4 is an essential component of the signaling process and its ability to so signal is one of its defining functions.

11. Lastly, researchers in the field of LPS signaling are well aware of the remaining members of the toll-like receptor family, generally, and are able to identify TLR-4 and its homologs using the structural and functional features shared by all TLR-4 polypeptides.

12. The examiner has rejected the claims on the grounds that practice of the invention as claimed would require undue experimentation. Particularly, the examiner asserts that the specification does not provide for methods of measuring LPS mediated responses other than through measuring altered expression of TLR-4 and therefore does not provide methods for

identification of compounds that may modulate LPS responses by any other mechanism than altering TLR-4 expression. I do not find this to be the case.

13. Contrary to the examiner's position, it is well within the skill of one in the field of endotoxin and cellular biology to screen for compounds that modulate the LPS responses through their action upon TLR-4 beyond up or down regulation of TLR-4 expression. The screening of candidate compounds for their effects upon protein action and interaction is routine in the field. In view of the contents of the application, such screening is not limited to those compounds that may alter TLR-4 expression. Indeed, the general expectation of researchers performing such screens is that they will produce small compounds that specifically alter the binding specificity, signaling capacity, or other functional property of the target protein, in this case, TLR-4.

14. The specification clearly sets forth assays of TLR-4 activity in the LPS response pathway that can be used by one of ordinary skill in the art to determine, without undue experimentation, whether or not such candidate compounds modulate the action of TLR-4 independently of any action upon TLR-4 expression. For example, such assays are described in the specification at pages 87-88. Furthermore, these and further assays are available through the general knowledge of one of skill in the field of endotoxin biology.

15. I expect, based upon my skill and training in the areas of endotoxin and cellular biology that an ordinary researcher in these areas would be able to routinely practice the claimed invention following the guidance provided in the application and using the knowledge generally available in endotoxin biology.

16. I declare that all statements made of my knowledge are true and all statements made on the information are believed to be true; and, further that these statements were made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under § 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this application or any patent issued thereupon.

Date: 9/26/02

David D. Chaplin

David D.Chaplin, M.D., Ph.D.

Exhibit D

CURRICULUM VITAE

Name:	David Dunbar Chaplin
Date of Birth:	August 28, 1952
Place of Birth:	London, England
Citizenship:	U.S.A.
Social Security Number:	067-38-2393
Home Address:	406 Wildwood Lane Indian Springs, AL 35124
Current Position:	Charles H. McCauley Professor and Chair Department of Microbiology University of Alabama at Birmingham 845 19th Street South, BBRB 276/11 Birmingham, AL 35294-2170
Undergraduate Education:	Harvard College Cambridge, Massachusetts A.B. June, 1973
Graduate Education:	Washington University St. Louis, Missouri M.D. May, 1980 Ph.D. May, 1980
Post-doctoral Training:	
1982-1984	Harvard Medical School, Department of Genetics Boston, Massachusetts, Fellow
1980-1982	University of Texas, Southwestern Medical School Parkland Memorial Hospital, Dallas, Texas Internal Medicine Residency
Academic Appointments:	
2001-present	Chairman, University of Alabama at Birmingham, Department of Microbiology, Birmingham, AL
2001-present	Senior Scientist, Comprehensive Cancer Center, University of Alabama at Birmingham
1995-2001	Associate Physician, Barnes-Jewish Hospital, University of Washington, St. Louis, MO
1995-2001	Professor, Washington University School of Medicine, Departments of Medicine, Genetics, and Molecular Microbiology, St. Louis, MO
1994-2001	Chief, Div. of Allergy and Immunology, Washington University School of Medicine, Department of Medicine

Academic Appointments (continued):

1992-1995	Assoc. Professor, Washington University School of Medicine, Department of Genetics, St. Louis, MO
1991-1995	Assoc. Professor, Washington University School of Medicine, Department of Medicine and Molecular Microbiology, St. Louis, MO
1989-1992	Asst. Professor, Washington University School of Medicine, Department of Genetics, St. Louis, MO
1984-1995	Assistant Physician, Barnes-Jewish Hospital, University of Washington, St. Louis, MO
1984-2001	Assoc. Investigator, Howard Hughes Medical Institute
1984-1991	Asst. Professor, Washington University School of Medicine, Dept. of Medicine and Molecular Microbiology, St. Louis, MO

Honors/Awards:

2001	Fellow, American Academy of Allergy, Asthma and Immunology
1997	Association of American Physicians
1995-1998	Councilor, American Society for Clinical Investigation
1993	Fellow, American Association for the Advancement of Science
1993	American Society for Clinical Investigation
1982-1984	Jane Coffin Childs Memorial Fund for Medical Research Fellowship
1980	Alpha Omega Alpha
1974-1980	Medical Scientist Trainee

Scientific Organizations:

2001-present	Secretary, American Academy of Allergy, Asthma and Immunology, Basic and Clinical Immunology Interest Section,
1994-2001	Associate Editor, Journal of Immunology
1993-present	International Cytokine Society
1991-present	American Academy of Allergy, Asthma and Immunology
1991-1996	Associate Editor, Diabetes
1989-1991	Associate Editor, The New Biologist
1989-present	American Society of Human Genetics
1986-present	American Association of Immunologists
1985-present	American Federation of Clinical Research
1984-present	American Association for the Advancement of Science

Keywords: Inflammatory Cytokines; TNF; IL-1; Asthma Pathogenesis; Lymphoid Tissue Development; Th Cell Function; Germinal Centers; Follicular Dendritic Cells

Publications:

1. Feagler, J.R., Tillack, T.W., Chaplin, D.D., and Majerus, P.W. (1974): The effects of thrombin on phytohemagglutinin receptor sites in human platelets. *J Cell Biol* 60:541-553.

2. Wedner, H.J., Chaplin, D.D., and Parker, C.W. (1977): Evidence for an early sulfhydryl reagent sensitive step during lymphocyte activation. In: Regulatory Mechanisms in Lymphocyte Activation, edited by D.O. Lucas, pp. 456-458, Academic Press, New York.

3. Chaplin, D.D., and Wedner, H.J. (1978): Inhibition of lectin-induced lymphocyte activation by diamide and other sulfhydryl reagents. *Cell Immunol* 36:303-311.

4. Chaplin, D.D., Wedner, H.J., and Parker, C.W. (1979): Protein phosphorylation in human peripheral blood lymphocytes: Subcellular distribution and partial characterization of adenosine 3', 5'-monophosphate-dependent protein kinase. *Biochem J* 182:525-536.

5. Chaplin, D.D., Wedner, H.J., and Parker, C.W. (1979): Protein phosphorylation in human peripheral blood lymphocytes: Phosphorylation of endogenous plasma membrane and cytoplasmic proteins. *Biochem J* 182:537-546.

6. Chaplin, D.D., Wedner, H.J., and Parker, C.W. (1980): Protein phosphorylation in human peripheral blood lymphocytes: Mitogen-induced increases in protein phosphorylation in intact lymphocytes. *J Immunol* 124:2390-2398.

7. Chaplin, D.D., Wedner, H.J., and Parker, C.W. (1980): Protein phosphorylation and lymphocyte activation. In: Biological Basis of Immunodeficiency, edited by E.W. Gelfand and H.-M. Dosch, pp. 269-281, Raven Press, New York.

8. Udey, M.C., Chaplin, D.D., Wedner, H.J., and Parker, C.W. (1980): Early activation events in lectin-stimulated human lymphocytes: Evidence that wheat germ agglutinin and mitogenic lectins cause similar early changes in lymphocyte metabolism. *J Immunol* 125:1544-1550.

9. Chaplin, D.D., Woods, D.E., Whitehead, A.S., Goldberger, G., Colten, H.R., and Seidman, J.G. (1983): Molecular map of the murine S region. *Proc Natl Acad Sci U S A* 80:6947-6951.

10. Chaplin, D.D., Sackstein, R., Perlmutter, D.H., Weis, J.H., Kruse, T.A., Coligan, J., Colten, H.R., and Seidman, J.G. (1984): Expression of hemolytically active murine fourth component of complement in transfected L cells. *Cell* 37:569-576.

11. Buse, J.B., Chaplin, D.D., Ben-Nun, A., Klein, K.A., Eisenbarth, G.S., Seidman, J.G., and Jackson, R.A. (1984): Class I, II, and III major histocompatibility complex gene polymorphisms in BB rats. *Diabetalogia* 27:77-79.

12. Weis, J.H., Nelson, D.L., Przyborski, M.J., Chaplin, D.D., Mulligan, R.C., Housman, D.E., and Seidman, J.G. (1984): Eukaryotic chromosome transfer: Linkage of the murine major histocompatibility complex to an inserted dominant selectable marker. *Proc Natl Acad Sci U S A* 81:4879-4884.

13. White, P.C., Chaplin, D.D., Weis, J.H., Dupont, B., New, M.I., and Seidman, J.G. (1984): Two steroid 21-hydroxylase genes are located in the murine S region. *Nature* 312:465-467.

14. White, P.C., Grossberger, D., Onufer, B., Chaplin, D.D., New, M.I., Dupont, B., and Strominger, J.L. (1985): Two genes encoding steroid 21-hydroxylase are located near the genes encoding the fourth component of complement in man. *Proc Natl Acad Sci U S A* 82:1089-1093.

15. Perlmutter, D.H., Colten, H.R., Grossberger, D., Strominger, J.L., Seidman, J.G., and Chaplin, D.D. (1985): Expression of complement proteins C2 and Factor B in transfected L cells. *J Clin Invest* 76:1449-1454.

16. Chaplin, D.D. (1985): Molecular organization and in vitro expression of murine class III genes. *Immunol Rev* 87:61-80.

17. Parker, K.L., Chaplin, D.D., Wong, M., Seidman, J.G., Smith, J.A., and Schimmer, B.P. (1985): Expression of murine 21-hydroxylase in mouse adrenal glands and in transfected Y1 adrenocortical tumor cells. *Proc Natl Acad Sci U S A* 82:7860-7864.

18. Buse, J.B., Rifai-Haddad, R., Lees, S., Taniguchi, H., Chaplin, D.D., Milford, E.M., Seidman, J.G., Eisenbarth, G.S., and Jackson, R.A. (1985): Major histocompatibility complex restriction fragment length polymorphisms define three diabetogenic haplotypes in BB and BBN rats. *J Exp Med* 162:444-458.

19. Chaplin, D.D., Galbraith, L.G., Seidman, J.G., White, P.C., and Parker, K.L. (1986): Nucleotide sequence analysis of murine 21-hydroxylase genes: mutations affecting gene expression. *Proc Natl Acad Sci U S A* 83:9601-9605.

20. Parker, K.L., Chaplin, D.D., Wong, M., Seidman, J.G., and Schimmer, B.P. (1986): Molecular analysis of 21-hydroxylase gene expression in mouse adrenal cells. *Endocr Res* 12:409-427.

21. Parker, K.L., Schimmer, B.P., Chaplin, D.D., and Seidman, J.G. (1986): Characterization of a regulatory region of the steroid 21-hydroxylase gene. *J Biol Chem* 261:15353-15355.

22. Hansbrough, J.R., Wedner, H.J., and Chaplin, D.D. (1987): Anaphylaxis to intravenous furosemide. *J Allergy Clin Immunol* 80:538-541.

23. Holers, V.M., Chaplin, D.D., Leykam, J.F., Gruner, B.A., Kumar, V., and Atkinson, J.P. (1987): Human complement C3b/C4b receptor (CR1) mRNA polymorphism that correlates with the CR1 allelic molecular weight polymorphism. *Proc Natl Acad Sci U S A* 84:2459-2463.

24. Graham, M.Y., Otani, T., Boime, I., Olson, M.V., Carle, G.F., and Chaplin, D.D. (1987): Cosmid mapping of the human chorionic gonadotropin β subunit genes by field-inversion gel electrophoresis. *Nucleic Acids Res* 15:4437-4448.

25. Fuhlbrigge, R.C., Chaplin, D.D., Kiely, J.-M., and Unanue, E.R. (1987): Regulation of interleukin 1 gene expression by adherence and lipopolysaccharide. *J Immunol* 138:3799-3802.

26. Unanue, E.R., Weaver, C.T., Fuhlbrigge, R.C., Kiely, J.-M., Chaplin, D.D.(1987): Membrane IL-1: a key protein in antigen presentation. *Annales de l'Institut Pasteur/Immunology* 138:489-492.

27. D'Eustachio, P., Jadidi, S., Fuhlbrigge, R.C., Gray, P.W., and Chaplin, D.D. (1987): Interleukin-1 α and β genes: linkage on chromosome 2 in the mouse. *Immunogenetics* 26:339-343.

28. Kemp, M.E., Atkinson, J.P., Skanes, V.M., Levine, R.P., and Chaplin, D.D. (1987): Deletion of C4A genes in patients with systemic lupus erythematosus. *Arthritis Rheum* 30:1015-1022.

29. Chou, H.S., Nelson, C.A., Godambe, S.A., Chaplin, D.D., and Loh, D.Y. (1987): Germline organization of the murine T-cell receptor β-chain genes. *Science* 238:545-548.

30. Chaplin, D.D., and Kemp, M.E. (1987): The major histocompatibility complex and autoimmunity. In, The Year in Immunology, 1986/87, vol. 3, ed. Cruse, J.M., Karger, Basel, pp. 179-198.

31. Chaplin, D.D., Fuhlbrigge, R.C., Jadidi, S., Sheehan, K.C.F., Schreiber, R.D., Gray, P.W., Unanue, E.R., and D'Eustachio, P. (1988): Restriction fragment length polymorphisms and linkage of murine IL-1 α and β genes on chromosome 2. In, Progress in Leukocyte Biology, Vol. 8, "Monokines and Other Non-lymphocytic Cytokines" eds. Powanda, M.C., Oppenheim, J.J., Kluger, M.J., and Dinarello, C.A., Alan R. Liss, New York, pp. 41-46.

32. Otani, T., Otani, F., Krych, M., Chaplin, D.D., and Boime, I. (1988): Identification of a promoter region in the CGβ gene cluster. *J Biol Chem* 263:7322-7329.

33. Fuhlbrigge, R.C., Sheehan, K.C.F., Schreiber, R.D., Chaplin, D.D., and Unanue, E.R. (1988): Monoclonal antibodies to murine interleukin-1α: production, characterization, and inhibition of membrane-associated interleukin-1 activity. *J Immunol* 141:2643-2650.

34. Fuhlbrigge, R.C., Fine, S.M., Unanue, E.R., and Chaplin, D.D. (1988): Expression of membrane interleukin-1 by fibroblasts transfected with murine pro-interleukin-1α cDNA. *Proc Natl Acad Sci USA* 85:5649-5653.

35. Vik, D.P., Keeney, J.B., Munoz-Canoves, P., Chaplin, D.D., and Tack, B.F. (1988): Structure of the murine complement factor H gene. *J Biol Chem* 263:16720-16724.

36. Fuhlbrigge, R.C., Hogquist, K.A., Unanue, E.R., and Chaplin, D.D. (1989): Molecular Biology and Genetics of Interleukin-1. In, The Year in Immunology, 1988, vol. 5, ed. Cruse, J.M. and Lewis, R.E., Karger, Basel, pp. 21-37.

37. Barnum, S.R., Kristensen, T., Chaplin, D.D., Seldin, M.F., and Tack, B.F. (1989): Molecular analysis of the murine C4b-binding protein gene. Chromosome assignment and partial gene organization. *Biochemistry* 28:8312-8317.

38. Storb, U., Engler, P., Hagman, J., Gollahon, K., Manz, J., Roth, P., Rudin, C., Doglio, L., Hackett, J., Haasch, D., Chaplin, D.D., Lo, D., and Brinster, R. (1989): Control of expression of immunoglobulin genes. *Progress in Immunology* 7:316-323.

39. Vik, D.P., Munoz-Canoves, P., Chaplin, D.D., and Tack, B.F. (1989): Factor H. *Curr Top Microbiol Immunol* 153:147-162.

40. Boime, I., Otani, T., Otani, F., and Chaplin, D.D. (1989): Regulation of the chorionic gonadotrophin β subunit genes. *Reprod Fertil Suppl* 37:1-10.

41. Amiguet, P., D'Eustachio, P., Kristensen, T., Wetsel, R.A., Saris, C.J.M., Hunter, T., Chaplin, D.D., and Tack, B.F. (1990): Structure and chromosome assignment of the murine p36 (calpactin-I heavy-chain) gene. *Biochemistry* 29:1226-1232.

42. Vik, D.P., Munoz-Canoves, P., Kozono, H., Martin, L.G., Tack, B.F., and Chaplin, D.D. (1990): Identification and sequence analysis of four complement factor H-related transcripts in mouse liver. *J Biol Chem* 265:3193-3201.

43. Rosenwasser, T.A., Hogquist, K.A., Nothwehr, S.F., Bradford-Goldberg, S., Olins, P.O., Chaplin, D.D., and Gordon, J.I. (1990): Compartmentalization of mammalian proteins produced in Escherichia-coli. *J Biol Chem* 265:13066-13073.

44. Hagman, J., Rudin, C.M., Haasch, D., Chaplin, D.D., and Storb, U. (1990): A novel enhancer in the immunoglobulin λ locus is duplicated and functionally independent of NFκ B. *Genes Dev* 4:978-992.

45. Kulczycki, A., Webber, J., Soares, H.A., Onken, M.D., Thompson, J.A., Chaplin, D.D., Loh, D.Y., and Tillinghast, J.P. (1990): Genomic organization of mouse Fcγ receptor genes. *Proc Natl Acad Sci U S A* 87:2856-2860.

46. Webb, G.C., and Chaplin, D.D. (1990): Genetic variability at the human tumor necrosis factor α and β loci. In, Molecular and Cellular Biology of Cytokines, eds. Oppenheim, J.J., Powanda, M.C., Kluger, M.J., and Dinarello, C.A., Alan R. Liss, New York, pp 31-35.

47. Webb, G.C., and Chaplin, D.D. (1990): Genetic variability at the human tumor necrosis factor loci. *J Immunol* 145:1278-1285.

48. Wroblewski, J.M., Kaminsky, S.G., Milisauskas, V.K., Pittman, A.M., Chaplin, D.D., Spies, T., and Nakamura, I. (1990): The B144-H-2D^b interval and the location of a murine homologue of the human D6S81E locus. *Immunogenetics* 32:200-204.

49. Howard, A.D., Kostura, M.J., Thornberry, N., Ding, G.J.F., Limjuco, G., Weidner, J., Salley, J.P., Hogquist, K.A., Chaplin, D.D., Mumford, R.A., Schmidt, J.A., and Tocci, M.J. (1991): IL-1-converting enzyme requires aspartic acid residues for processing of the IL-1β precursor at two distinct sites and does not cleave 31-kDa IL-1α. *J Immunol* 147:2964-2969.

50. Bronson, S.K., Pei, J., Taillon-Miller, P., Chorney, M.J., Geraghty, D.E., and Chaplin, D.D. (1991): Isolation and characterization of yeast artificial chromosome clones linking the HLA-B and HLA-C loci. *Proc Natl Acad Sci U S A* 88:1676-1680.

51. Hogquist, K.A., Nett, M.A., Sheehan, K.C.F., Pendleton, K.D., Schreiber, R.D., and Chaplin, D.D. (1991): Generation of monoclonal antibodies to murine IL-1β and demonstration of IL-1 in vivo. *J Immunol* 146:1534-1540.

52. Kozono, H., Bronson, S.K., Taillon-Miller, P., Moorti, M.K., Jamry, I., and Chaplin, D.D. (1991): Molecular linkage of the HLA-DR, HLA-DQ, and HLA-DO genes in yeast artificial chromosomes. *Genomics* 11:577-586.

53. Hogquist, K.A., Unanue, E.R., and Chaplin, D.D. (1991): Release of interleukin-1 from mononuclear phagocytes. *J Immunol* 147:2181-2186.

54. Hogquist, K.A., Nett, M.A., Unanue, E.R., and Chaplin, D.D. (1991): Interleukin-1 is processed and released during apoptosis. *Proc Natl Acad Sci U S A* 88:8485-8489.

55. Parimoo, S., Patanjali, S.R., Shukla, H., Chaplin, D.D., and Weissman, S.M. (1991): cDNA selection: efficient PCR approach for the selection of cDNAs encoded in large chromosomal DNA fragments. *Proc Natl Acad Sci U S A* 88:9623-9627.

56. Chaplin, D.D., and Hogquist, K.A. (1992): Interactions between TNF and Interleukin-1. In, Tumor Necrosis Factors: The Molecules and their Emerging Role in Medicine, ed. Beutler, B., Raven Press, Ltd., New York, chap. 13, pp. 197-220.

57. Domalik, L.J., Chaplin, D.D., Kirkman, M.S., Wu, R.C., Seldin, M.F., Howard, T.A., and Parker, K.L. (1992): Different isozymes of mouse 11β-hydroxylase produce mineralocorticoids and glucocorticoids. *Mol Endocrinol* 5:1853-1861.

58. Chaplin, D.D., and Tillinghast, J.P. (1992): The molecular basis of immune recognition. In, Allergy: Theory and Practice, 2nd edition, eds. Korenblat, P.E., and Wedner, H.J., W. B. Saunders, Philadelphia, pp. 31-60.

59. Bora, N.S., Chaplin, D.D., and Atkinson, J.P. (1992): Restriction fragment length polymorphisms of proteins of the complement system. In, Manual of Clinical Laboratory Immunology, 4th edition, Rose, N.E., de Macario, E.C., Fahey, J.L., Friedman, H., and Penn, G.M., eds., American Society for Microbiology, Washington, D.C., pp. 153-155.

60. Geraghty, D.E., Pei, J., Lipsky, B., Hansen, J., Taillon-Miller, P., Bronson, S.K., and Chaplin, D.D. (1992): Cloning and physical mapping of the HLA class I region spanning the HLA-E to HLA-F interval using yeast artificial chromosomes. *Proc Natl Acad Sci U S A* 89:2669-2673.

61. Nett, M.A., Cerretti, D.P., Berson, D.R., Seavitt, J., Gilbert, D.J., Jenkins, N.A., Copeland, N.G., Black, R.A., and Chaplin, D.D. (1992): Molecular cloning of the murine interleukin-1β converting enzyme cDNA. *J Immunol* 149:3254-3259.

62. Chaplin, D.D., and Brownstein, B.H. (1992): Strategies for screening YAC (yeast artificial chromosome) libraries and for analysis of YAC clones. In Current Protocols in Molecular Biology, eds. Ausubel F., Brent, R., Kingston, R., Moore, D., Seidman, J., Smith, J., and Struhl, K. Current Protocols, New York, units 6.9 and 6.10.

63. Demmer, L.A., and Chaplin, D.D. (1993): Simultaneous transfer of four functional genes from the HLA class II genes region into mammalian cells by fusion with yeast spheroplasts carrying an artificial chromosome. *J Immunol* 150:5371-5378.

64. Hunt, C.R., Gasser, D.L., Chaplin, D.D., Pierce, J.C., and Kozak, C.A. (1993): Chromosomal localization of five murine Hsp70 gene family members: Hsp70-1, Hsp70-2, Hsp70-3, Hsc70t, and Grp78. *Genomics* 16:193-198.

65. Udalova, I.A., Nedospasov, S.A., Webb, G.C., Chaplin, D.D., and Turetskaya, R.L. (1993): Highly informative typing of the human TNF locus using six adjacent polymorphic markers. *Genomics* 16:180-186.

66. Dawson, J., Rordorf-Adam, C., Geiger, T., Towbin, H., Kunz, S., Nguyen, H., Zingel, O., Chaplin, D., and Vosbeck, K. (1993): Interleukin-1 (IL-1) production in a mouse tissue chamber model of inflammation. 1. Development and initial characterization of the model. *Agents Actions* 38:247-254.

67. Dawson, J., Rordorf-Adam, C., Geiger, T., Towbin, H., Kunz, S., Nguyen, H., Zingel, O., Chaplin, D., and Vosbeck, K. (1993): Interleukin-1 (IL-1) production in a mouse tissue chamber model of inflammation. 2. Identification of (tissue) macrophages as the IL-1 producing cells and the effect of anti-inflammatory drugs. *Agents Actions* 38:255-264.

68. Godambe, S.A., Chaplin, D.D., and Bellone, C.J. (1993): Regulation of IL-1 gene expression: differential responsiveness of murine macrophage lines. *Cytokine* 5:327-335.

69. Zhou, Y., and Chaplin, D.D. (1993): Identification in the HLA class I region of a gene expressed late in keratinocyte differentiation. *Proc Natl Acad Sci U S A* 90:9470-9474.

70. Wei, H., Fan, W.-F., Xu, H., Parimoo, S., Shukla, H., Chaplin, D.D., and Weissman, S.M. (1993): Genes in one megabase of the HLA class I region. *Proc Natl Acad Sci U S A* 90:11870-11874.

71. Gasser, D.L., Sternberg, N.L., Pierce, J.C., Goldner-Sauve, A., Feng, J., Haq, A.K., Spies, T., Hunt, C., Buetow, K.H., and Chaplin, D.D. (1994): P1 and cosmid clones define the organization of 280 kb of the mouse *H-2* complex containing the *Cps-1* and *Hsp70* loci. *Immunogenetics* 39:48-55.

72. Godambe, S.A., Chaplin, D.D., Takova, T., and Bellone, C.J. (1994): Upstream NFIL-6-like site located within a DNase I hypersensitivity region mediates LPS-induced transcription of the murine interleukin-1 β gene. *J Immunol* 153:143-152.

73. De Togni, P., Goellner, J., Ruddle, N.H., Streeter, P.R., Fick, A., Mariathasan, S., Smith, S.C., Carlson, R., Shornick, L.P., Strauss-Schoenberger, J., Russell, J.H., Karr, R.W., and Chaplin, D.D. (1994): Abnormal development of peripheral lymphoid organs in mice deficient in lymphotoxin. *Science* 264:703-707.

74. Godambe, S.A., Chaplin, D.D., Takova, T., and Bellone, C.J. (1994): An NFIL-6 sequence near the transcriptional initiation site is necessary for the lipopolysaccharide induction of murine interleukin-1β (IL-1β). *DNA Cell Biol* 13:561-569.

75. Krishnan, B.R., and Chaplin, D.D. (1994): Fluorescent automated sequencing of supercoiled high molecular weight double-stranded DNA. *Biotechniques* 17:854-857.

76. Krishnan, B.R., Jamry, I., Berg, D.E., Berg, C.M., and Chaplin, D.D. (1995): Construction of a genomic DNA 'Feature Map' by sequencing from nested deletions: application to the HLA class I region. *Nucleic Acids Res* 23:117-122.

77. Godambe, S.A., Chaplin, D.D., Takova, T., and Bellone, C.J. (1995): Molecular dissection of the murine IL-1β promoter. *Am J Ther* 2:677-686.

78. Godambe, S.A., Chaplin, D.D., Takova, T., and Bellone, C.J. (1995): A novel cis-acting element required for lipopolysaccharide-induced transcription of the murine IL-1β gene. *Mol Cell Biol* 15:112-119.

79. Chaplin, D.D., and Zhou, Y. (1995): HLA-Linked Skin Disease: Classical HLA genes or novel genes within HLA? *J Invest Dermatol* 104:37S.

80. Krishnan, B.R., Jamry, I., and Chaplin, D.D. (1995): Feature mapping of the HLA Class I region - localization of the POU5F1 and TCF19 genes. *Genomics* 30:53-58.

81. Min, J., Shukla, H., Kozono, H., Bronson, S., Weissman, S., and Chaplin, D.D. (1995): A novel Creb family gene telomeric of HLA-DRA in the HLA complex. *Genomics* 30:149-156.

82. Mariathasan, S., Matsumoto, M., Baranyay, F., Nahm, M., Kanagawa, O., and Chaplin, D.D. (1995): Absence of lymph nodes in lymphotoxin-α (LT-α)-deficient mice is due to abnormal organ development, not defective lymphocyte migration. *J Inflamm* 45:72-78.

83. LaBlanca, F., Krishnan, B.R., Chaplin, D.D., Berg, D.E, and Berg, C.E. (1995): Restriction map of a 35-kb HLA fragment constructed by nested deletion 'drop-out' mapping. *Gene* 164:335-339.

84. Nett-Fiordalisi, M., Thomaselli, K., Russell, J.H., and Chaplin, D.D. (1995): Macrophage apoptosis in the absence of active interleukin-1β converting enzyme. *J Leukoc Biol* 58:717-724.

85. Krishnan, B.R., Young, A., and Chaplin, D.D. (1996): An improved and universal T7-end primer for fluorescent sequencing of nested deletion derivatives obtained using 'Deletion Factory' vectors. *Focus* 18:25-26.

86. Matsumoto, M., Mariathasan, S., Nahm, M.H., Baranyay, F., Peschon, J.J., and Chaplin, D.D. (1996). Role of lymphotoxin and the type I TNF receptor in the formation of germinal centers. *Science* 271:1289-1291.

87. Molina, H., Holers, V.M., Li, B., Fang, Y.-F., Mariathasan, S., Goellner, J., Strauss-Schoenberger, J., Karr, R.W., and Chaplin, D.D. (1996): Markedly impaired humoral immune response in mice deficient in complement receptors 1 and 2. *Proc Natl Acad Sci U S A* 93:3357-3361.

88. Shornick, L.P., De Togni, P., Mariathasan, S., Goellner, J., Strauss-Schoenberger, J., Karr, R.W., Ferguson, T., and Chaplin, D.D. (1996): Mice deficient in IL-1β manifest impaired contact hypersensitivity to trinitrochlorobenzene. *J Exp Med* 183:1427-1436.

89. Matsumoto, M., Lo, S.F., Carruthers, C.J.L., Min, J., Mariathasan, S., Huang, G., Plas, D.R., Martin, S.M., Geha, R.S., Nahm, M.H., and Chaplin, D.D. (1996): Affinity maturation without germinal centers in lymphotoxin-α (LTα) deficient mice. *Nature* 382:462-466.

90. Fu, Y.-X., Huang, G., Matsumoto, M., Molina, H., and Chaplin, D.D. (1997): Independent signals regulate development of primary and secondary follicle structure in spleen and mesenteric lymph node. *Proc Natl Acad Sci U S A* 94:5739-5743.

91. Matsumoto, M., Fu, Y.-X., Molina, H., and Chaplin, D.D. (1997): Lymphotoxin-α-deficient and TNF receptor-I-deficient mice define developmental and functional characteristics of germinal centers. *Immunol Rev* 156:137-144.

92. Fu, Y., Molina, H., Matsumoto, M., Huang, G., Min, J., and Chaplin, D.D. (1997): Lymphotoxin-α (LTα) supports development of splenic follicular structure that is required for IgG responses. *J Exp Med* 185:2111-2120.

93. Matsumoto, M., Fukuda, W., Circolo, A., Goellner, J., Strauss-Schoenberger, J., Wang, X., Fujita, S., Hidvegi, T., Chaplin, D.D., and Colten, H.R. (1997): Abrogation of the alternative complement pathway by targeted deletion of murine factor B. *Proc Natl Acad Sci U S A* 94: 8720-8725.

94. Matsumoto, M., Fu, Y.-X., Molina, H., Huang, G., Kim, J., Thomas, D.A., Nahm, M.H., and Chaplin, D.D. (1997): Distinct roles of lymphotoxin α and the type I tumor necrosis factor (TNF) receptor in the establishment of follicular dendritic cells from non-bone marrow-derived cells. *J Exp Med* 186:1997-2004.

95. Fu, Y., Huang, G., Wang, Y., and Chaplin, D.D. (1998): B lymphocytes induce the formation of follicular dendritic cell clusters in a lymphotoxin-α-dependent fashion. *J Exp Med* 187:1009-1018.

96. Giegel, D.A., and Chaplin, D.D. (1998): Targets in cytokine activation. *Agents Actions Suppl* 49:1-3.

97. Chaplin, D.D., and Fu, Y.-X. (1998): Cytokine regulation of secondary lymphoid organ development. *Curr Opin Immunol* 10:289-297.

98. Randolph, D.A., Carruthers, C.J.L., Szabo, S., Murphy, K.M., and Chaplin, D.D. (1999): Modulation of airway inflammation by passive transfer of allergen-specific Th1 and Th2 cells in a mouse model of asthma. *J Immunol* 162:2375-2383.

99. Fu, Y.-X., and Chaplin, D.D. (1999): Development and maturation of secondary lymphoid tissues. *Annu Rev Immunol* 17:399-433.

100. Iizuka, K., Chaplin, D.D., Wang, Y., Wu, Q., Pegg, L.E., Yokoyama, W.M., and Fu, Y.-X. (1999): Requirement for membrane lymphotoxin in natural killer cell development. *Proc Natl Acad Sci U S A* 96:6336-6340.

101. Grossman, W. J., Verbsky, J. W., Yang, L., Berg, L. J., Fields, L. E., Chaplin, D. D., and Ratner, L. (1999): Dysregulated myelopoiesis in mice lacking Jak3. *Blood* 94:932-939.

102. Randolph, D.A., Stephens, R., Carruthers, C.J.L., and Chaplin, D.D. (1999): Cooperation between Th1 and Th2 cells in a murine model of eosinophilic airway inflammation. *J Clin Invest* 104:1021-1029.

103. Randolph, D.A., Huang, G., Carruthers, C.J.L., Bromley, L.E., and Chaplin, D.D. (1999): The Role of CCR7 in T(H)1 and T(H)2 Cell Localization and Delivery of B Cell Help in Vivo. *Science* 286:2159-2162.

104. Castro, M., Chaplin, D.D., Walter, M.J., and Holtzman, M.J. (2000): Could asthma be worsened by stimulating the T-helper type 1 immune response? *Am J Respir Cell Mol Biol* 22:143-146

105. Fu, Y.-X., Huang, G., Wang, Y., and Chaplin, D.D. (2000): Lymphotoxin-α-dependent spleen microenvironment supports the generation of memory B cells and is required for their subsequent antigen-induced activation. *J Immunol* 164:2508-2514.

106. Wang, Y., Huang, G., Wang, J., Molina, H., Chaplin, D. D., and Fu, Y.-X. (2000): Antigen persistence is required for somatic mutation and affinity maturation of immunoglobulin. *Eur J Immunol* 30:2226-2234.

107. Rennert, P. D., Hochman, P. S., Flavell, R. A., Chaplin, D. D., Jayaraman, S., Browning, J. L., and Fu, Y.-X. (2001): Essential role of lymph nodes in contact hypersensitivity revealed in lymphotoxin-α-deficient mice. *J Exp Med* 193:1227-1238.

108. Hussain, I., Randolph, D., Brody, S. L., Song, S. K., Hsu, A., Kahn, A. M., Chaplin, D. D., and Hamilos, D. L. (2001): Induction, distribution and modulation of upper airway allergic inflammation in mice. *Clin Exp Allergy* 31:1048-1059.

109. Mandik-Nayak, L., Huang, G., Sheehan, K.C.F., Erikson, J., and Chaplin, D.D. (2001): Signaling through TNF receptor p55 in TNF-α-deficient mice alters the CXCL13/CCL19/CCL21 ratio in the spleen and induces maturation and migration of anergic B cells into the B cell follicle. *J Immunol* 167:1920-1928.

110. Grayson, M. H., Chaplin, D. D., Karl, I. E., and Hotchkiss, R. S. (2001): Confocal fluorescent intravital microscopy of the murine spleen. *J Immunol Methods* 256:55-63.

111. Shornick, L.P., Bisarya, A.K., and Chaplin, D.D. (2001): IL-1β is essential for Langerhans cell activation and antigen delivery to lymph nodes during contact sensitization: evidence for a dermal source of IL-1β. *Cellular Immunol* 211:105-112.

112. Dube, P.H., Revell, P.A., Chaplin, D.D., Lorenz, R.G., and Miller, V.L. (2001): A role for IL-1α in inducing pathologic inflammation during bacterial infection. *Proc. Natl. Acad. Sci. USA* 98:10880-10885.

113. Mason, J.L., Suzuki, K., Chaplin, D.D., and Matsushima, G.K. (2001): Interleukin-1β promotes repair of the CNS. *J Neurosci 21*:7046-7052.

114. Byersdorfer, C.A., and Chaplin, D.D. (2001); Visualization of early APCT/T cell interactions in the mouse lung following intranasal challenge. *J Immunol* 167:6756-6764.

115. Verbsky, J.W., Randolph, D.A., Shornick, L.P., and Chaplin, D.D. (2002): Nonhematopoietic expression of Janus Kinase 3 is required for efficient recruitment of Th2 lymphocytes and eosinophils in OVA-induced airway inflammation. *J Immunol* 168:2475-2482.

116. Stephens, R., Eisenbarth, S.C., Chaplin, D.D. (2002): T helper type 1 cells in asthma: friend or foe? *Curr Opin Allergy Clin Immunol* 2:31-37.

Invited Lectures:

Jan. 26, 1984	The Royal Society of London, Biochemistry and Genetics of Complement: Cloning and expression of murine C4 and Slp.
Dec. 12, 1988	Univ. of Missouri, Dept. of Microbiology: Molecular immunology of Interleukin-1.
Dec. 17, 1991	Univ. of Texas Medical Branch at Galveston: Interleukin-1, a secreted cytokine?
Nov. 7, 1994	National Workshop on Alopecia Areata: HLA-linked skin disease: classical HLA genes or novel genes within HLA?
Jan. 31, 1995	Ohio State Univ.: Molecular Analysis of the HLA Complex.
Aug. 25, 1995	BASF BioResearch Corp: Gene Targeting to Define the Role of IL-1β *in vivo.*
Feb. 15, 1996	Barnes-Jewish Medical Grand Rounds: Gene Targeting to Define the *in Vivo* Functions of Cytokines
May 10, 1996	6th International Congress, TNF and Related Molecules, Rhodes, Greece: Lymphotoxin-α-Deficient and TNF-Receptor I-Deficient Mice Define Developmental and Functional Characteristics of Germinal Centers.
May 21, 1996	St. Louis Jewish Hospital Grand Rounds: Gene Targeting to Define the *in Vivo* Functions of Cytokines
Oct. 28, 1996	Chairman, Inflammation Research Association Conference Session: Targets and Cytokine Action
Dec. 16, 1996	University of Washington Immunology Program: Role of Lymphotoxin in Peripheral Lymphoid Tissue Development
Dec. 17, 1996	Immunex Corp.: Essential Role of IL-1β in Contact Hypersensitivity Responses
Feb. 13, 1997	Biogen Corp.: Role of Lymphotoxin in Peripheral Lymphoid Tissue Development
Mar. 20, 1997	New York University School of Medicine/Skirball Institute: Essential Role of Lymphotoxin in Peripheral Lymphoid Tissue Development
Apr. 11, 1997	University of Utah, Developmental Biology Program: Cytokine Signals for Lymphoid Tissue Development
May 21, 1997	Pfizer Corp.: Role of Lymphotoxin in Peripheral Lymphoid Tissue Development
May 22, 1997	Inflammation Research Association: Induction of IL-1 During Apoptosis

June 24, 1997	FASEB Conference on Autoimmunity: Cytokine Signals for Lymphoid issue Development
July 1, 1997	Gordon Conference: Lymphotoxin, a Primary Determinant of Lymphoid Tissue Structure
Oct.8, 1997	National Jewish Center for Immunology and Respiratory Diseases: Lymphotoxin, a Primary Determinant of Lymphoid Tissue Structure
Dec. 3, 1997	Duke University, Department of Immunology: Role of Lymphotoxin in Peripheral Lymphoid Tissue Development
Jan. 27, 1998	37th Midwinter Immunology Conference, Asilomar: Lymphotoxin-Dependent Signals Controlling Peripheral Lymphoid Tissue Development
Feb. 19, 1998	University of North Carolina, Department of Microbiology: Lymphotoxin-Dependent Signals Controlling Peripheral Lymphoid Tissue Development
Mar. 2, 1998	University of Rochester, Department of Pediatrics: Lymphotoxin-Dependent Signals Controlling Peripheral Lymphoid Tissue Development
May 20, 1998	7th International TNF Congress, Hyannis: Lymphotoxin-Dependent Signals Controlling Peripheral Lymphoid Tissue Development
June 23, 1998	FASEB Conference on Lymphocytes and Antibodies: TNF/LT Family Members as Signals for Lymphoid Tissue Development
June 26, 1998	International Union of Immunological Societies, Symposium on Primary Immunodeficiency Diseases: Cytokine Signals for the Development of Primary B Cell Follicle Structure
Sept. 9, 1998	St. Jude Children's Research Hospital, Department of Immunology: Lymphotoxin-Dependent Signals Controlling Peripheral Lymphoid Tissue Development
Oct. 27, 1998	International Cytokine Society, Jerusalem: Lymphotoxin-Dependent Signals Regulating Primary B Cell Follicle Structure and Function
Dec. 7, 1998	Washington University Center for Immunology Seminar: Signals Controlling Normal Lymphoid Tissue Structure and Function
Dec. 9, 1998	Wistar Institute: Lymphotoxin, a Major Determinant for Normal Secondary Lymphoid Tissue Development and Function
Jan. 26, 1999	Vanderbilt University, Department of Microbiology and Immunology: Signals Controlling Normal Lymphoid Tissue Structure and Function

Feb. 11, 1999	Keystone Conference: B Lymphocyte Biology and Disease TNF Family Members in Formation of Primary Lymphoid Follicles
Feb. 27, 1999	American Academy of Allergy, Asthma and Immunology, 55th Annual Meeting: Synergy of Th1 and Th2 Cells in Experimental Eosinophilic Airway Inflammation
Mar. 15, 1999	University of Toronto, Immunology Department Seminar Series: Cellular and Molecular Determinants of Peripheral Lymphoid Tissue Structure and Function
May 8, 1999	Nikolas Symposium, Athens, Greece: Cytokines and Lymphoid Tissue Development
Sept. 25, 1999	National Residency Education Program, American Association of Allergy, Asthma, and Immunology, St. Louis, MO: Allergy-Immunology: from Bench to Bedside.
Oct. 22, 1999	Allergy Abroad, Paris, France: Cooperation Between T Helper Cells in Allergic Airway Inflammation
Oct. 26, 1999	Allergy Abroad, Lyon, France: Control of Lymphocyte Movement and Function by Chemokines
Oct. 29, 1999	Allergy Abroad, Montpellier, France Organization and Function of Secondary Lymphoid Tissues
Nov. 9, 1999	Stanford University, Program in Immunology Seminar: Regulation of Lymphoid Tissue Structure and Function
Nov. 30, 1999	Kyoto University, Department of Molecular Genetics: Regulation of Lymphoid Tissue Structure and Function
Dec. 2, 1999	Kyoto, Japan, 29th Annual Meeting of the Japanese Society for Immunology, Symposium on Lymphocyte Development in Germinal Centers: Targeting within Secondary Lymphoid Tissues and Control of Antibody Responses
Apr. 5, 2000	University of Alabama at Birmingham, Department of Microbiology Regulation of Lymphoid Tissue Structure and Function
Apr. 17, 2000	NIAID/NCI Symposium: Cells of the Marginal Zone – Origins, Function and Neoplasia, Bethesda, MD: Regulation of secondary lymphoid tissue follicle structure and function by lymphotoxin
May 13, 2000	AAI Annual Meeting, Seattle, WA. Major Symposium Co-Chair: Molecular Mechanisms of Lymphoid Organogenesis. Regulation of secondary lymphoid tissue follicle structure and function by lymphotoxin
Aug. 19, 2000	Clinical Allergy for the Practicing Physician, St. Louis, MO. DNA Vaccines

Sept. 9, 2000	1st International Workshop on Nucleotides and Their Receptors in the Immune System, Ferrara, Italy Is apoptosis required for IL-1 action *in vivo*?
Oct. 3, 2000	Howard Hughes Medical Institute: Infection and Immunity Molecular Determinants of Spleen Follicle Structure and Function
Oct. 25, 2000	University of Iowa, Department of Microbiology Regulation of secondary lymphoid tissue follicle structure and function by lymphotoxin
Jan. 17, 2001	Albert Einstein College of Medicine, Division of Biological Sciences Seminar Series Molecular Determinants of Spleen Follicle Structure and Function
Mar. 12, 2001	Washington University Center for Immunology Seminar: Regulation of Secondary Lymphoid Tissue Structure and Function by Lymphotoxin and TNF
Mar. 18, 2001	57th Annual Meeting of the American Academy of Allergy, Asthma and Immunology, New Orleans, LA: Grand Seminar. Regulation of Secondary Lymphoid Tissue Structure and Function by Lymphotoxin
Apr. 19, 2001	New York University Immunology Program Seminar: Mechanisms Regulating Th2-dependent Inflammation in Peripheral Tissues
May 23, 2001	Mucosal Immunology at the 21st Century, Perdido Beach, AL: Plasticity of Secondary Lymphoid Tissue Structures
June 7, 2001	NIH/NIAID Asthma Center Directors Meeting, Bethesda, MD: Regulation of T Helper Cell Recruitment to Peripheral Tissues
July 23, 2001	11th International Congress of Immunology, Stockholm, Sweden: Symposium on Antigen Processing and Presentation at Mucosal Surfaces. Control of Lymphoid Tissue Structure and Function by LT and TNF
Nov. 6, 2001	EU and NIH Conference, Siena, Italy: Potential Impact of New Technologies on Vaccination in Early Life. Signals for Development of Secondary Lymphoid Organs
Dec. 5, 2001	British Society for Immunology Annual Congress, Harrogate, UK: Plenary Speaker. Recruitment of Th2 Cells to Peripheral Sites *in vivo*
Jan. 22, 2002	Department of Microbiology, University of Alabama at Birmingham: Recruitment of Th2 Cells to Peripheral Sites *in vivo*
Feb. 8, 2002	9th International Conference on Lymphocyte Traffic and Homeostasis, Newport Beach, CA: Structural Elements Regulating Lymphocyte Trafficking to and in the Spleen
Mar. 2, 2002	58th Annual Meeting of the American Academy of Allergy, Asthma and Immunology,

New York, NY: Role of Inflammation in Recruitment of Th2 Lymphocytes to the Lung

June 9, 2002 FASEB Conference, Anatomy of the Immune Response *in vivo*, Snowmass, CO: Lymphocyte Trafficking Patterns in the Spleen

Exhibit E.

TABLE 7-3 Intracellular or Extrac llular Growth Preference Relativ to Eukaryotic Cells

Category	Bacterial Pathog n
Obligate intracellular	*Rickettsia* spp *Coxiella burnetii* *Chlamydia* spp
Facultative intracellular	*Salmonella* spp *Shigella* spp *Legionella pneumophila* Invasive *Escherichia coli* *Neisseria* spp *Mycobacterium* spp *Listeria monocytogenes* *Bordetella pertussis*
Predominantly extracellular	*Mycoplasma* spp *Pseudomonas aeruginosa* Enterotoxigenic *Escherichia coli* *Vibrio cholerae* *Staphylococcus aureus* *Streptococcus pyogenes* *Haemophilus influenzae* *Bacillus anthracis*

bacterial surface factors that mediate invasion are not known in most instances, and often, multiple gene products are involved. Some *Shigella* invasion factors are encoded on a 140 megadalton plasmid, which, when conjugated into *E coli*, gives these noninvasive bacteria the capacity to invade cells. Other invasion genes have also recently been identified in Salmonella and *Yersinia pseudotuberculosis*. The mechanisms of invasion of *Rickettsia*, and *Chlamydia* species are not well known.

Capsules and Other Surface Components

Bacteria have evolved numerous structural and metabolic virulence factors that enhance their survival rate in the host. Capsule formation has long been recognized as a protective mechanism for bacteria (see Ch. 2). Encapsulated strains of many bacteria (e.g., pneumococci) are more virulent and more resistant to phagocytosis and intracellular killing than are nonencapsulated strains. Organisms that cause bacteremia (e.g., *Pseudomonas*) are less sensitive than many other bacteria to killing by fresh human serum containing complement components, and consequently are called serum resistant. Serum resistance may be related to the amount and composition of capsular antigens as well as to the structure of the lipopolysaccharide. The relationship between surface structure and virulence is important also in *Borrelia* infections. As the bacteria encounter an increasing specific immune response from the host, the bacterial surface antigens are altered by mutation, and the progeny, which are no longer recognized by the immune response, express renewed virulence. *Salmonella typhi* and some of the paratyphoid organisms carry a surface antigen, the *Vi antigen*, thought to enhance virulence. This antigen is composed of a polymer of galactosamine and uronic acid in 1,4-linkage. Its role in virulence has not been defined, but antibody to it is protective.

Some bacteria and parasites have the ability to survive and multiply inside phagocytic cells. A classic example is *Mycobacterium tuberculosis*, whose survival seems to depend on the structure and composition of its cell surface. The parasite *Toxoplasma gondii* has the remarkable ability to block the fusion of lysosomes with the phagocytic vacuole. The hydrolytic enzymes contained in the lysosomes are unable, therefore, to contribute to the destruction of the parasite. The mechanism(s) by which bacteria such as *Legionella pneumophila, Brucella abortus,* and *Listeria monocytogenes* remain unharmed inside phagocytes are not understood.

Endotoxins

Endotoxin is comprised of toxic lipopolysaccharide components of the outer membrane of Gram-negative bacteria (see Ch. 2). Endotoxin exerts profound biologic effects on the host and may be lethal. Because it is omnipresent in the environment, endotoxin must be removed from all medical supplies destined for injection or use during surgical procedures. The term endotoxin was coined in 1893 by Pfeiffer to distinguish the class of toxic substances released after lysis of bacteria from the toxic substances (exotoxins) secreted by bacteria. Few, if any, other microbial products have been as extensively studied as bacterial endotoxins. Perhaps it is appropriate that a molecule with such important biologic effects on the host, and one produced by so many bacterial pathogens, should be the subject of intense investigation.

Structure of Endotoxin

Figure 7-5 illustrates the basic structure of endotoxin. Endotoxin is a molecular complex of lipid and polysaccharide; hence, the alternate name lipopolysaccharide. The complex is secured to the outer membrane by ionic and hydrophobic forces, and its strong negative charge is neutralized by Ca^{2+} and Mg^{2+} ions.

The structure of endotoxin molecules from *Salmonella* spp and *E coli* is known in detail. Enough data on endotoxin from other Gram-negative organisms have been gathered to reveal a common pattern with genus and species diversity. Although all endotoxin molecules are similar in chemical

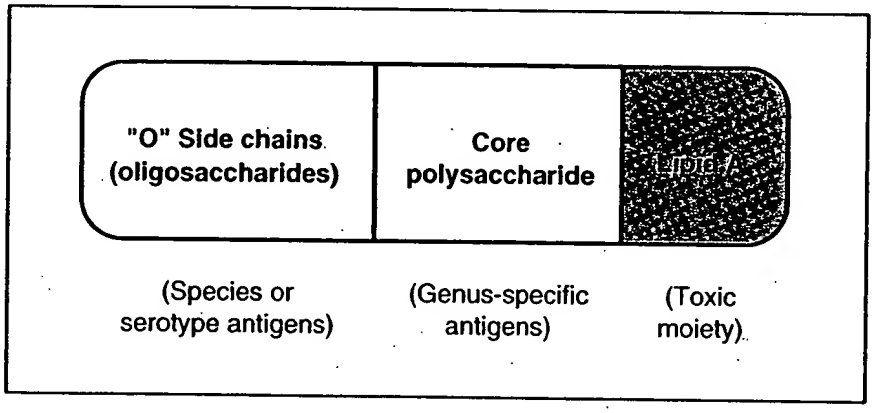

FIGURE 7-5 Basic structure of endotoxin (lipopolysaccharide) from Gram-negative bacteria.

structure and biologic activity, some diversity has evolved. Purified endotoxin appears as large aggregates. The molecular complex can be divided into three regions (Fig. 7-5): (1) the O-specific chains, which consist of a variety of repeating oligosaccharide residues, (2) the core polysaccharide that forms the backbone of the macromolecule, and (3) lipid A, composed usually of a glucosamine disaccharide with attached long-chain fatty acids and phosphate. The polysaccharide portions are responsible for antigenic diversity, whereas the lipid A moiety confers toxicity. Dissociation of the complex has revealed that the polysaccharide is important in solubilizing the toxic lipid A component, and in the laboratory it can be replaced by carrier proteins (e.g., bovine serum albumin).

Members of the family Enterobacteriaceae exhibit O-specific chains of various lengths, whereas *N gonorrhoeae, N meningitidis,* and *B pertussis* contain only core polysaccharide and lipid A. Some investigators working on the latter forms of endotoxin prefer to call them lipooligosaccharides to emphasize the chemical difference from the endotoxin of the enteric bacilli. Nevertheless, the biologic activities of all endotoxin preparations are essentially the same, with some being more potent than others.

Biologic Activity of Endotoxin

The biologic effects of endotoxin have been extensively studied. Purified lipid A (conjugated to bovine serum albumin) and endotoxin elicit the same biologic responses. Table 7-4 lists some of the biologic effects of endotoxin. The more pertinent toxic effects include pyrogenicity, leukopenia followed by leukocytosis, complement activation, depression in blood pressure, mitogenicity, induction of prostaglandin synthesis, and hypothermia. These events can culminate in sepsis and lethal shock. However, it should be noted from Table 7-4 that not all effects of endotoxin are necessarily detrimental; several induce responses potentially beneficial to the host, assuming the stimulation is not excessive. These include:

1. mitogenic effects on B lymphocytes that increase resistance to viral and bacterial infections
2. induction of gamma interferon production by T lymphocytes, which may enhance the antiviral state, promote rejection of tumor cells, and activate macrophages and natural killer cells
3. activation of the complement cascade with the formation of C3a and C5a
4. induction of the formation of interleukin-1 by macrophages and interleukin-2 and other mediators by T lymphocytes.

Current research focuses on exploiting some of the potential beneficial effects of "nontoxic" endotoxin derivatives and holds promise for development of future treatment regimens for stimulating the immune response. For example, the toxicity of endotoxin is largely attributed to lipid A, attached to a polysaccharide carrier. The toxicity of lipid A is markedly reduced after hydrolysis of a phosphate group or deacylation of one or more fatty acids from the lipid A molecule. Clinical trials are in progress to test a monophosphoryl lipid A for its potential of inducing low dose tolerance to endotoxin. Tolerance to endotoxin can be achieved by pretreatment of an animal with low doses of endotoxin or a detoxified lipid A derivative before challenge with high doses of endotoxin. Experimental studies have demonstrated that induction of tolerance to endotoxin reduces the dangerous effects of endotoxin. It is hoped that these relatively nontoxic lipid A derivatives may be useful in reducing the severity of bacterial sepsis in which bacterial endotoxin produces a life-threatening clinical course.

TABLE 7-4 Multiple Biologic Activiti s Exhibited by the Lipid A Compon nt of Endotoxin

Pyrogenicity
Leukopenia, leukocytosis
Complement activation
Depression of blood pressure
Hageman factor activation
Platelet activation
Induction of plasminogen activator
Bone marrow necrosis
Hypothermia in mice
Lethal toxicity in mice
Shwartzman reaction
Induction of prostaglandin synthesis
Limulus lysate gelation
*Induction of nonspecific resistance to infection
*Induction of endotoxin tolerance
*Adjuvant activity
*Mitogenic activity for lymphocytes
*Macrophage activation
*Induction of interferon synthesis
*Induction of tumor necrosis factor synthesis

*Potentially beneficial stimulatory effects of endotoxin in low doses.

Endotoxin, which largely accumulates in the liver following injection of a sublethal dose by the intravenous route, can be devastating because of its ability to affect a variety of cell and host proteins. Kupffer cells, granulocytes, macrophages, platelets, and lymphocytes all have a cell receptor on their surface called CD14, which binds endotoxin. Endotoxin binding to the CD14 receptor on macrophages is enhanced by interaction with a host protein made in the liver (i.e., LPS-binding protein). The extent of involvement of each cell type probably depends on the level of endotoxin exposure. The effects of endotoxin on such a wide variety of host cells result in a complex array of host responses that can culminate in the serious condition gram-negative sepsis, which often leads to shock and death. The effects of endotoxin on host cells are known to stimulate prostaglandin synthesis and to activate the kallikrein system, the kinin system, the complement cascade via the alternative pathway, the clotting system, and the fibrinolytic pathways. When these normal host systems are activated and operate out of control, it is not surprising that endotoxin can be lethal. Although it is difficult to comprehend the mechanisms of all the cell responses and the myriad sequelae of the cell mediators released rather indiscriminately in the host following exposure to endotoxin, it does seem clear that the host cellular response to endotoxin, rather than a direct toxic effect of endotoxin, plays the major role in causing tissue damage (Fig. 7-3).

Detection of Endotoxin in Medical Solutions

Endotoxin is omnipresent in the environment. It is found in most deionized-water lines in hospitals and laboratories, for example, and affects virtually every biologic assay system ever examined. It tends to be a scapegoat for all biologic problems encountered in the laboratory, and, many times, this reputation is deserved. Because of its pyrogenic and destructive properties, extreme care must be taken to avoid exposing patients to medical solutions containing endotoxin. Even though all supplies should be sterile, solutions for intravenous administration can become contaminated with endotoxin-containing bacteria after sterilization as a result of improper handling. Furthermore, water used in the preparation of such solutions must be filtered through ion exchange resins to remove endotoxin, because it is not removed by either autoclave sterilization or filtration through bacterial membrane filters. If endotoxin-containing solutions were used in such medical procedures as renal dialysis, heart bypass machines, blood transfusions, or surgical lavage, the patient would suffer immediate fever accompanied by a rapid and possibly lethal alterations in blood pressure.

Solutions for human or veterinary use are prepared under carefully controlled conditions to ensure sterility and to remove endotoxin. Representative samples of every manufacturing batch are checked for endotoxin by one of two procedures: the *Limulus* lysate test or the rabbit pyrogenicity test. The rabbit pyrogenicity test is based on the exquisite sensitivity of rabbits to the pyrogenic effects of endotoxin. A sample of the solution to be tested usually is injected intravenously into the ear veins of adult rabbits while the rectal temperature of the animal is monitored. Careful monitoring of the temperature responses provides a sensitive and reliable indicator of the presence of endotoxin and, importantly, one measure of the safety of the solution for use in patients.

The *Limulus* lysate test is more common and less expensive. This test, which is based on the ability of endotoxin to induce gelation of lysates of amebocyte cells from the horseshoe crab *Limulus polyphemus*, is simple, fast, and sensitive (about 1 ng/ml). It is so sensitive, however, that trace quantities of endotoxin in regular deionized water often obscure the results. It can be used for rapid detection of certain Gram-negative infections (e.g., of cerebrospinal fluid); however, blood contains inhibitors that prevent gelation. Test kits are

commercially available. The amebocyte is the sole phagocytic immune cell of the horseshoe crab, and the gelation reaction is believed to be involved in sequestering invading Gram-negative bacteria.

Exotoxins

Exotoxins, unlike the lipopolysaccharide endotoxin, are protein toxins released from viable bacteria. They form a class of poisons that is among the most potent, per unit weight, of all toxic substances. Most of the higher molecular-sized exotoxin proteins are heat labile; however, numerous low molecular-sized exotoxins are heat-stable peptides. Unlike endotoxin, which is a structural component of all Gram-negative cells, exotoxins are produced by some members of both Gram-positive and Gram-negative genera. The functions of these exotoxins for the bacteria are usually unknown, and the genes for most can be deleted with no noticeable effect on bacterial growth. In contrast to the extensive systemic and immune-system effects of endotoxin on the host, the site of action of most exotoxins is more localized and is confined to particular cell types or cell receptors. Tetanus toxin, for example, affects only internuncial neurons. In general, exotoxins are excellent antigens that elicit specific antibodies called antitoxins. Not all antibodies to exotoxins are protective, but some react with important binding sites or enzymatic sites on the exotoxin, resulting in complete inhibition of the toxic activity (i.e., neutralization).

Exotoxins can be grouped into several categories (e.g., neurotoxins, cytotoxins, and enterotoxins) based on their biologic effect on host cells. Neurotoxins are best exemplified by the toxins produced by *Clostridium* spp, for example, the botulinum toxin formed by *C botulinum*. This potent neurotoxin acts on motor neurons by preventing the release of acetylcholine at the myoneural junctions, thereby preventing muscle excitation and producing flaccid paralysis. The cytotoxins constitute a larger, more heterogeneous grouping with a wide array of host cell specificities and toxic manifestations. One cytotoxin is diphtheria toxin, which is produced by *Corynebacterium diphtheriae*. This cytotoxin inhibits protein synthesis in many cell types by catalyzing the ADP-ribosylation of elongation factor II, which blocks elongation of the growing peptide chain.

Enterotoxins stimulate hypersecretion of water and electrolytes from the intestinal epithelium and thus produce watery diarrhea. Some enterotoxins are cytotoxic (e.g., shiga-like enterotoxin from E coli), while others perturb eukaryotic cell functions and are cytotonic (e.g., cholera toxin). Enterotoxins also can disturb normal smooth muscle contraction, causing abdominal cramping and decrease transit time for water absorption in the intestine. Enterotoxigenic *E coli* and *V cholerae* produce diarrhea after attaching to the intestinal mucosa, where they elaborate enterotoxins. Neither pathogen invades the body in substantial numbers, except in the case of *E coli* species that have acquired an invasion plasmid. Importantly, cholera toxin and *E coli* heat-labile enterotoxins I and II cause ADP-ribosylation of cell proteins in a manner similar to diphtheria toxin, except that the primary target is the regulatory protein (G_s) of adenylate cyclase, resulting in increased levels of cyclic 3',5'-adenosine monophosphate (cAMP) (see Ch. 25). In contrast, the organisms responsible for shigellosis (*Shigella dysenteriae, S boydii, S flexneri,* and *S sonnei*) penetrate the mucosal surface of the colon and terminal ileum to proliferate and cause ulcerations that bleed into the intestinal lumen. Despite causing extensive ulceration of the mucosa, the pathogens rarely enter the bloodstream. The Shiga enterotoxin produced by *Shigella* species and the Shiga-like enterotoxin elaborated by many isolates of *E coli* inhibit protein synthesis in eukaryotic cells. It is not clear how this cytotoxic enterotoxin causes hypersecretion of water and electrolytes from the intestinal epithelium. These enterotoxins differ from those secreted by *V cholerae* and *E coli* in that the Shiga toxins are cytotoxic and lethal, whereas the cholera toxin-like enterotoxins are not. The latter enterotoxins cause no structural damage to cells, and are described as cytotonic. The ensuing inflammatory response to the invading bacteria and/or their toxins appears to activate neurologic control mechanisms (e.g., prostaglandins, serotonin) that normally regulate water and electrolyte transport.

Siderophores

Both animals and bacteria require iron for metabolism and growth, and the control of this limited resource is often used as a tactic in the conflict between pathogen and host. Animals have evolved mechanisms of "withholding" iron from tissue fluids in an attempt to limit the growth of invading bacteria. Although blood is a rich source of iron, this iron is not readily available to bacteria since it is not free in solution. Most of the iron in blood is bound either to hemoglobin in erythrocytes or to transferrin in plasma. Similarly, the iron in milk and other secretions (e.g., tears, saliva, bronchial mucus, bile, and gastrointestinal fluid) is bound to lactoferrin. Some bacteria express receptors for eukoyotic iron-binding proteins (e.g., transferrin-binding outer membrane proteins on the surface of *Neisserira spp*). Via these specialized receptors iron acquisition is facilitated, providing the esssential element for bacterial growth.

Other bacteria have evolved elaborate mechanisms to extract the iron from host proteins (Fig. 7-6). Siderophores are substances produced by many bacteria (and some plants)

Exhibit E

CERTIFICATE OF MAILING
37 C.F.R §1.8

I hereby certify that this correspondence is being deposited with the U.S. Postal Service as First Class Mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231, on the date below:

10/23/2003
Date

Mark B. Wilson

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Beutler, *et al.*

Serial No.: 09/396,985

Filed: September 15, 1999

For: LPS-RESPONSE GENE COMPOSITIONS AND METHODS

Group Art Unit: 1646

Examiner: Basi, N.

Atty. Dkt. No.: UTSD:602

DECLARATION OF DAVID D. CHAPLIN, M.D., Ph.D.

I, David D. Chaplin, hereby declare as follows:

1. I am a U.S. citizen residing at 406 Wildwood Lane, Indian Springs, Alabama 34124. I am the Charles H. McCauley Professor and Chairman of the Department of Microbiology at the University of Alabama at Birmingham. I have extensive experience in the study of cellular responses to endotoxins. References containing examples of my work are included in my *Curriculum Vitae*. A copy of my *Curriculum Vitae* is attached as Appendix 2.

2. I understand that the present invention relates to methods for screening for modulators of TLR-4 mediated responses to lipopolysaccharide (LPS) mediated responses. The methods involve the use of a TLR-4 polypeptide and the measurement of LPS mediated responses, themselves mediated by TLR-4, in the presence and absence of a putative modulatory compound.

3. I understand that the Examiner has rejected several claims of the application on the grounds that the term "small molecule inhibitor" is not clear so as to allow the metes and bounds of the claim to be determined. I do not find this to be the case.

4. I have reviewed the final Office Action dated December 18, 2002, the specification of the application, and the pending claims. In light of these documents, and my knowledge of the field of endotoxins and cellular biology, I make the following statements.

5. The term "small molecule inhibitor" is in common use in the fields of endotoxins and cellular biology. A skilled researcher would understand that, in the context of the instant specification and claims, the term "small molecule inhibitor" describes low molecular weight, non-peptide inhibitors. This definition is accepted in the art. For a search in PubMed, a database of relevant scientific literature, revealed 61 references in which the words "small molecule" and "inhibitor" appears in the title alone (Appendix 1). This evidences that skilled researchers understand the meaning of the term "small molecule inhibitor."

6. I expect, based upon my skill and training in the areas of endotoxins and cellular biology that those of skill in those fields understand the definition of the term "small molecule inhibitor" as it is used in the specification and claims.

7. I declare that all statements made of my knowledge are true and all statements made on the information are believed to be true; and, further that these statements were made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under § 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this application or any patent issued thereupon.

Date: 10/22/03

David D.Chaplin, M.D., Ph.D.

Exhibit F

standard deviations are variance weighted. Rhythmic traces are those with any period estimate in the circadian range, 15–35 h, as described[8]. All rhythm assays were conducted at 22 °C.

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1. Thomas, B. & Vince-Prue, D. *Photoperiodism in Plants* (Academic, San Diego, 1997).
2. Carré, I. A. Day-length perception and the photoperiodic regulation of flowering in *Arabidopsis*. *J. Biol. Rhythms* 16, 416–424 (2001).
3. Alabadi, D. *et al.* Reciprocal regulation between *TOC1* and *LHY/CCA1* within the *Arabidopsis* circadian clock. *Science* 293, 880–883 (2001).
4. Wang, Z. & Tobin, E. M. Constitutive expression of the *CIRCADIAN CLOCK ASSOCIATED 1 (CCA1)* gene disrupts circadian rhythms and suppresses its own expression. *Cell* 93, 1207–1217 (1998).
5. Putterill, J., Robson, F., Lee, K., Simon, R. & Coupland, G. The *CONSTANS* gene of *Arabidopsis* promotes flowering and encodes a protein showing similarities to zinc finger transcription factors. *Cell* 80, 847–857 (1995).
6. Koornneef, M., Hanhart, C. J. & van der Veen, J. H. A genetic and physiological analysis of late flowering mutants in *Arabidopsis thaliana*. *Mol. Gen. Genet.* 229, 57–66 (1991).
7. Samach, A. & Coupland, G. Time measurement and the control of flowering in plants. *BioEssays* 22, 38–47 (2000).
8. Dowson-Day, M. J. & Millar, A. J. Circadian dysfunction causes aberrant hypocotyl elongation patterns in *Arabidopsis*. *Plant J.* 17, 63–71 (1999).
9. Hicks, K. A. *et al.* Conditional circadian dysfunction of the *Arabidopsis early-flowering 3* mutant. *Science* 274, 790–792 (1996).
10. Zagotta, M. T. *et al.* The *Arabidopsis ELF3* gene regulates vegetative photomorphogenesis and the photoperiodic induction of flowering. *Plant J.* 10, 691–702 (1996).
11. McWatters, H. G., Bastow, R. M., Hall, A. & Millar, A. J. The *ELF3* zeitnehmer regulates light signalling to the circadian clock. *Nature* 408, 716–720 (2000).
12. Liu, X. L., Covington, M. F., Fankhauser, C., Chory, J. & Wagner, D. R. *ELF3* encodes a circadian clock-regulated nuclear protein that functions in an *Arabidopsis* PHYB signal transduction pathway. *Plant Cell* 13, 1293–1304 (2001).
13. Covington, M. F. *et al.* *ELF3* modulates resetting of the circadian clock in *Arabidopsis*. *Plant Cell* 13, 1305–1315 (2001).
14. Hicks, K. A., Albertson, T. M. & Wagner, D. R. *EARLY FLOWERING 3* encodes a novel protein that regulates circadian clock function and flowering in *Arabidopsis*. *Plant Cell* 13, 1281–1292 (2001).
15. Reed, J. W. *et al.* Independent action of *ELF3* and *phyB* to control hypocotyl elongation and flowering time. *Plant Physiol.* 122, 1149–1160 (2000).
16. Bell-Pedersen, D., Crosthwaite, S. K., Lakin-Thomas, P. L., Merrow, M. & Okland, M. The Neurospora circadian clock: simple or complex? *Phil. Trans. R. Soc. Lond. B* 356, 1697–1709 (2001).
17. Aronson, B. D., Johnson, K. A. & Dunlap, J. C. Circadian clock locus frequency: protein encoded by a single open reading frame defines period length and temperature compensation. *Proc. Natl Acad. Sci. USA* 91, 7683–7687 (1994).
18. Strayer, C. *et al.* Cloning of the *Arabidopsis* clock gene *TOC1*, an autoregulatory response regulator homolog. *Science* 289, 768–771 (2000).
19. Feldmann, K. A. T-DNA insertion mutagenesis in *Arabidopsis*: mutational spectrum. *Plant J.* 1, 71–82 (1991).
20. Onouchi, H., Igeno, M. I., Perilleux, C., Graves, K. & Coupland, G. Mutagenesis of plants overexpressing *CONSTANS* demonstrates novel interactions among *Arabidopsis* flowering-time genes. *Plant Cell* 12, 885–900 (2000).
21. Suarez-Lopez, P. *et al.* *CONSTANS* mediates between the circadian clock and the control of flowering in *Arabidopsis*. *Nature* 410, 1116–1120 (2001).
22. Davis, S. J., Bhoo, S. H., Durski, A. M., Walker, J. M. & Vierstra, R. D. The heme-oxygenase family required for phytochrome chromophore biosynthesis is necessary for proper photomorphogenesis in higher plants. *Plant Physiol.* 126, 656–669 (2001).
23. Kardailsky, I. *et al.* Activation tagging of the floral inducer *FT*. *Science* 286, 1962–1965 (1999).
24. Bognar, L. K. *et al.* The circadian clock controls the expression pattern of the circadian input photoreceptor, phytochrome B. *Proc. Natl Acad. Sci. USA* 96, 14652–14657 (1999).
25. Carré, I. A. & Kay, S. A. Multiple DNA–protein complexes at a circadian-regulated promoter element. *Plant Cell* 7, 2039–2051 (1995).

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Competing interests statement

The authors declare that they have no competing financial interests.

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Protective role of phospholipid oxidation products in endotoxin-induced tissue damage

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Lipopolysaccharide (LPS), an outer-membrane component of Gram-negative bacteria, interacts with LPS-binding protein and CD14, which present LPS to toll-like receptor 4 (refs 1, 2), which activates inflammatory gene expression through nuclear factor κB (NFκB) and mitogen-activated protein-kinase signalling[3,4]. Antibacterial defence involves activation of neutrophils that generate reactive oxygen species capable of killing bacteria[5]; therefore host lipid peroxidation occurs, initiated by enzymes such as NADPH oxidase and myeloperoxidase[6]. Oxidized phospholipids are pro-inflammatory agonists promoting chronic inflammation in atherosclerosis[7]; however, recent data suggest that they can inhibit expression of inflammatory adhesion molecules[8]. Here we show that oxidized phospholipids inhibit LPS-induced but not tumour-necrosis factor-α-induced or interleukin-1β-induced NFκB-mediated upregulation of inflammatory genes, by blocking the interaction of LPS with LPS-binding protein and CD14. Moreover, in LPS-injected mice, oxidized phospholipids inhibited inflammation and protected mice from lethal endotoxin shock. Thus, in severe Gram-negative bacterial infection, endogenously formed oxidized phospholipids may function as a negative feedback to blunt innate immune responses. Furthermore, identified chemical structures capable of inhibiting the effects of endotoxins such as LPS could be used for the development of new drugs for treatment of sepsis.

Lipids containing polyunsaturated fatty acids such as 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphorylcholine (PAPC) are especially prone to oxidative modification. Oxidation of PAPC results in generation of a mixture of oxidation products (OxPAPC), some of which stimulate adhesion of monocytes to endothelial cells and induce expression of inflammatory genes in endothelial cells[8–10]. Thus, a role for oxidized phospholipids as culprits in chronic inflammation was suggested. On the other hand, we have shown that OxPAPC inhibited neutrophil adhesion to endothelial cells induced by LPS[8].

Here we show that OxPAPC blocked LPS-induced upregulation of the inflammatory adhesion molecules E-selectin, ICAM-1 and VCAM-1 on human umbilical-vein endothelial cells (HUVEC) (Fig. 1a). The inhibitory effect of OxPAPC was concentration-dependent, with half-maximal inhibition observed at 10 $\mu g\,ml^{-1}$ OxPAPC (Fig. 1b). The effects of LPS at concentrations as high as 500 $ng\,ml^{-1}$ were inhibited by 50 $\mu g\,ml^{-1}$ OxPAPC. Because expression of E-selectin, ICAM-1 and VCAM-1 is NFκB-dependent, we examined the influence of OxPAPC on signalling events at different levels of the NFκB cascade. We found that OxPAPC inhibited LPS-induced activation of a 5 × NFκB–luciferase reporter construct (Fig. 1c), binding of p65 to its DNA consensus site (Fig. 1d), and phosphorylation and degradation of IκBα (Fig. 1e).

NFκB is activated during inflammation by various receptors including the LPS toll-like receptor 4 (TLR4), the interleukin 1 (IL-1) receptor, and the tumour-necrosis factor-α (TNFα) receptor. In contrast to LPS, TNFα-induced phosphorylation and degra-

dation of IκBα were not significantly inhibited by OxPAPC (Fig. 1e). Moreover, OxPAPC blocked LPS-induced but not TNFα-induced activation of p38 mitogen-activated protein (MAP) kinase (Fig. 1e). Signalling events downstream of the IL-1 receptor and TLR4 converge on MyD88, which binds to the cytosolic domains of the receptors and couples them to downstream signalling cascades[4]. However, effects of IL-1β on expression of E-selectin were not inhibited by OxPAPC (Fig. 1f). Thus, these data indicate that the inhibitory effect of OxPAPC was specific for LPS and occurred upstream of MyD88, apparently at the level of LPS recognition by cellular or soluble receptor(s).

Activation of innate immune responses by LPS can be inhibited by various soluble molecules that bind LPS and neutralize its activity. These include certain plasma proteins, lipoproteins and lipids[11–14] and inhibitors structurally related to polymyxin B[15]. Alternatively, the action of LPS is inhibited by lipid-A-like LPS antagonists, which form inactive complexes with TLR4 or its accessory proteins[16]. Both the inhibitory action of LPS-binding substances as well as lipid-A-like LPS antagonists can be overcome by high LPS concentrations[17]. We found, however, that increasing LPS concentrations could not overcome the inhibitory effect of OxPAPC (Fig. 2a). Moreover, LPS-trapping substances are known to inhibit LPS-induced clotting activity of the *Limulus* amoebocyte lysate (LAL), while several lipid-A-like LPS antagonists activate clotting[18]. OxPAPC neither stimulated nor inhibited LPS-induced clotting activity of the LAL (Fig. 2b). Although serum also contains LPS-binding activity, increasing concentrations of serum did not mimic the inhibitory action of OxPAPC on E-selectin expression induced by LPS (Fig. 2c). These data indicate that OxPAPC inhibits LPS action by a mechanism different from known LPS antagonists. We hypothesized that OxPAPC could interfere with recognition of LPS by LPS-binding protein (LBP) and CD14, both presenting LPS to TLR4 (ref. 2)

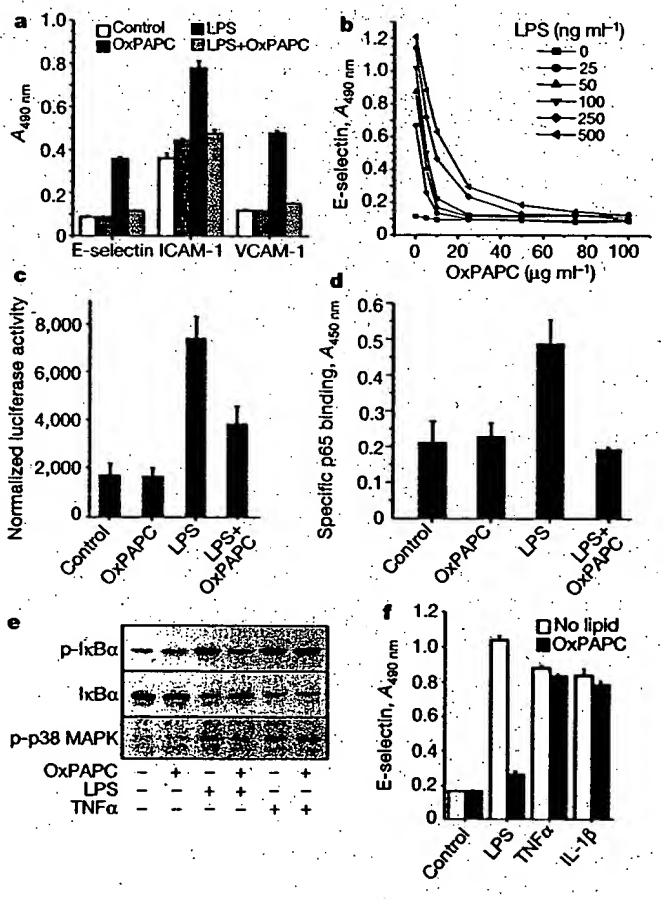

Figure 1 OxPAPC selectively inhibits LPS-induced intracellular signalling and expression of inflammatory adhesion molecules in HUVEC. **a**, OxPAPC inhibits LPS-induced upregulation of E-selectin, ICAM-1 and VCAM-1. **b**, The inhibitory effect of OxPAPC on LPS-induced E-selectin expression is concentration-dependent. **c**, OxPAPC inhibits LPS-induced activation of a 5 × NFκB–luciferase reporter. **d**, OxPAPC inhibits LPS-induced DNA-binding activity of p65. 'Specific' binding represents the difference between the binding in the absence or presence of excess of soluble consensus oligonucleotide. **e**, OxPAPC blocks LPS-induced phosphorylation (p) and degradation of IκBα and p38 MAP kinase phosphorylation. **f**, OxPAPC inhibits the action of LPS, but not of IL-1β and TNFα. HUVEC were stimulated with LPS (300 ng ml^{-1}), TNFα (20 U ml^{-1}) or IL-1β (10 ng ml^{-1}) in the presence or absence of 50 μg ml^{-1} OxPAPC. **a**–**f**, See Methods for additional experimental details. $A_{490\ nm}$, absorbance at 490 nm.

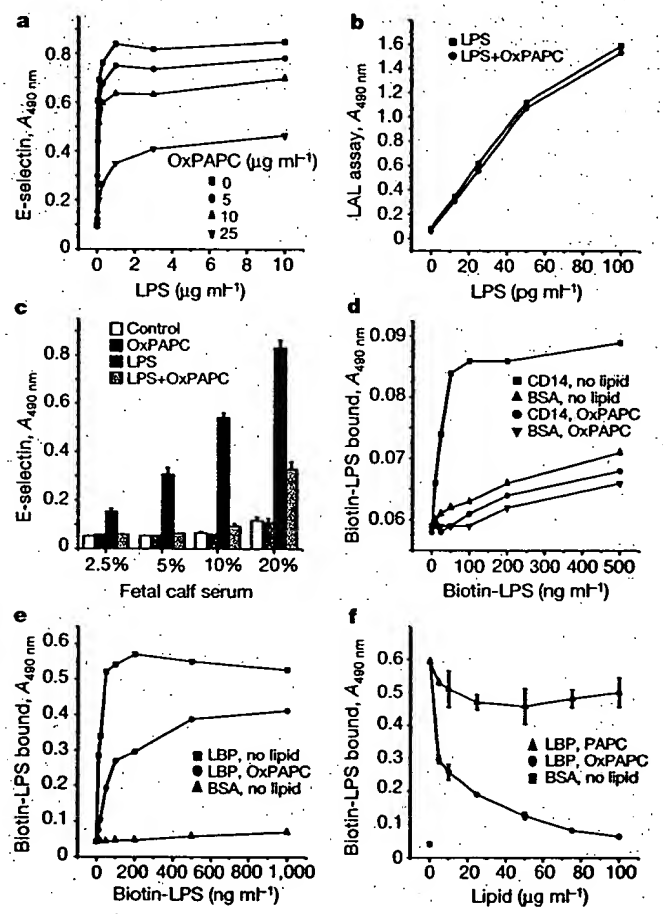

Figure 2 OxPAPC inhibits effects of LPS by blocking interactions of LPS with LBP and CD14. **a**, Increasing concentrations of LPS do not overcome the inhibitory activity of OxPAPC. HUVEC were stimulated with increasing concentrations of LPS in the presence of indicated concentrations of OxPAPC. E-selectin ELISA was performed as described in Methods. **b**, OxPAPC does not interfere with the LAL endotoxin assay. **c**, LPS-induced expression of E-selectin is not inhibited by high concentrations of serum. HUVEC were stimulated with LPS in the presence or absence of OxPAPC in medium 199 supplemented with indicated concentrations of bovine calf serum. **d**, OxPAPC inhibits binding of biotin-LPS to immobilized CD14. Binding and detection of biotinylated LPS to CD14- or BSA-coated microwell plates were performed as described in the Methods. Where indicated, the incubation medium contained 50 μg ml^{-1} OxPAPC. **e**, OxPAPC inhibits binding of biotin-LPS to immobilized LBP. **f**, Concentration dependence of the inhibition of LPS/LBP binding by OxPAPC. Microwells containing immobilized LBP were treated with indicated concentrations of sonicated native or oxidized PAPC and 100 ng ml^{-1} biotin-LPS. Detection of bound biotin-LPS was performed as described in Methods.

and being critical for LPS signalling in endothelial cells[19,20]. Apart from LPS, CD14 and LBP have been shown to bind phospholipids catalysing their transfer between cells and lipoproteins[21,22]. Indeed, OxPAPC inhibited binding of LPS to immobilized CD14 (Fig. 2d). OxPAPC also inhibited binding of LPS to immobilized LBP (Fig. 2e). The inhibitory action was concentration-dependent and specific for oxidized PAPC, whereas native PAPC was not effective (Fig. 2f).

Next we examined structural requirements for lipids to inhibit effects of LPS. We found that the inhibitory activity was specific for oxidized phospholipids because other lipids that are potentially present in OxPAPC preparations and known to exert a variety of

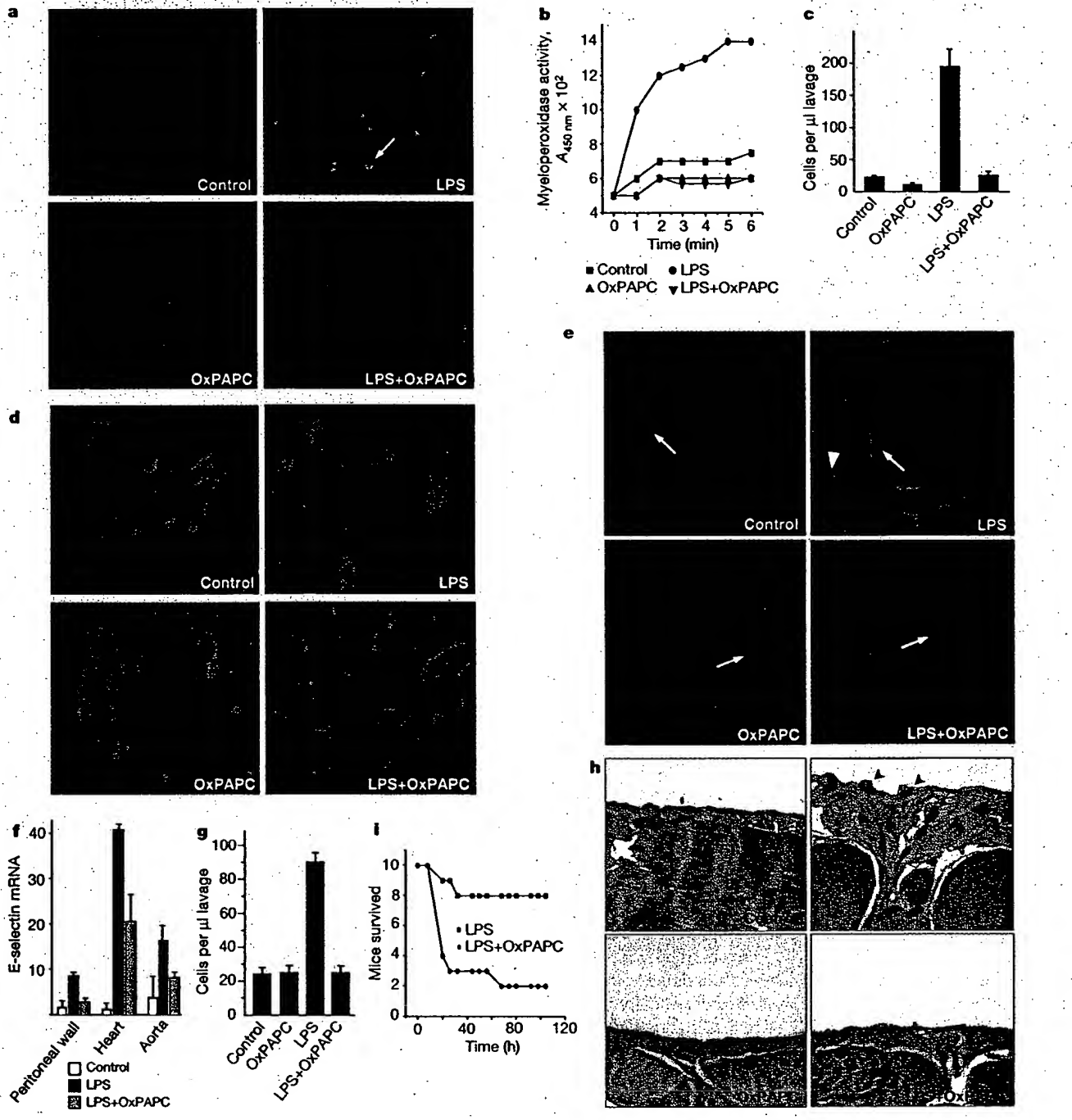

Figure 3 Oxidized phospholipids inhibit effects of endotoxin *in vivo*. **a**, OxPAPC inhibits LPS-induced accumulation of leukocytes reactive for myeloperoxidase in mouse skin. Arrow indicates myeloperoxidase-expressing leukocyte. **b**, Myeloperoxidase activity in the skin of LPS/OxPAPC-treated mice. **c**, OxPAPC inhibits LPS-induced accumulation of leukocytes in the air pouch model of inflammation. **d**, OxPAPC inhibits LPS-induced nuclear translocation of p65 in the air pouch wall. We note colocalization of p65 (green) with DAPI-stained nuclei (blue) in LPS-treated cells. **e**, OxPAPC inhibits LPS-induced VCAM-1 expression in the air pouch wall. Arrow indicates endothelial cell, arrowhead indicates myofibroblasts of the pouch wall. **f**, OxPAPC inhibits induction of E-selectin mRNA by LPS. Expression of E-selectin mRNA in heart, peritoneal wall and aorta was measured after injection of 50 μg of LPS intraperitoneally with or without 200 μg of OxPAPC in 0.9% saline. After 3 h, animals were killed, and the tissues were processed for E-selectin mRNA quantification. **g**, OxPAPC inhibits LPS-induced extravasation of white blood cells into the peritoneum. **h**, OxPAPC inhibits LPS-induced oedema formation in the peritoneal wall. Staining is with haematoxylin–eosin. Arrowheads indicate inflammatory cells. **i**, OxPAPC increases survival in mice treated with lethal doses of LPS. **a**–**i**, See Methods for experimental details.

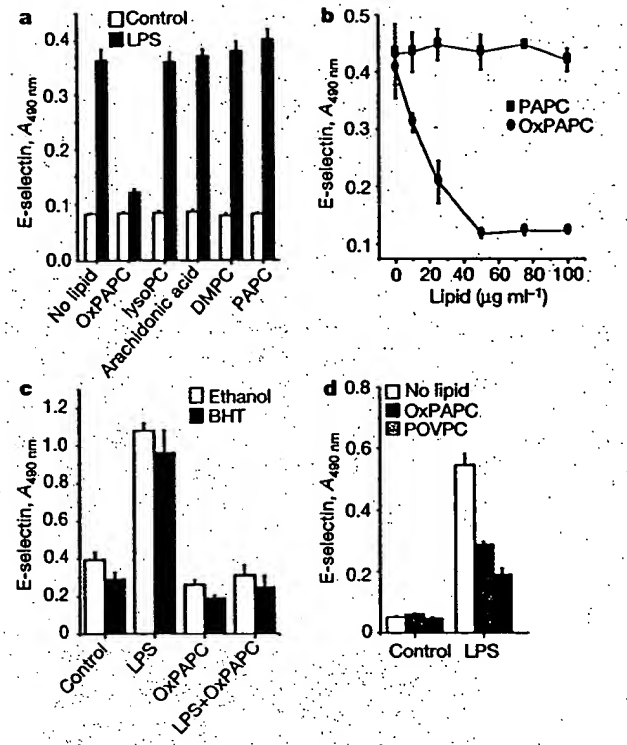

Figure 4 The anti-endotoxin activity of OxPAPC is not mimicked by other lipids, crucially depends on OxPAPC oxidation, and is not mediated by free radicals. **a**, The inhibitory effect of OxPAPC is not mimicked by other lipids. HUVEC were incubated with the indicated lipids (30 μg ml^{-1}) and 300 ng ml^{-1} LPS. **b**, Oxidation of PAPC is a prerequisite for its anti-endotoxin activity. HUVEC were treated with indicated concentrations of sonicated native or oxidized PAPC and 300 ng ml^{-1} LPS. **c**, Inhibitory action of OxPAPC is not rescued by the free-radical scavenger butylated hydroxytoluene (BHT). HUVEC were stimulated with LPS/OxPAPC in the presence of 25 μM BHT or 0.1% ethanol (vehicle). **d**, POVPC inhibits LPS-induced upregulation of E-selectin. HUVEC were treated with POVPC or OxPAPC (each at 20 μg ml^{-1}) and LPS (300 ng ml^{-1}). **a–d**, After 4 h, surface expression of E-selectin was determined by cell ELISA as described in Methods.

biological effects, namely arachidonic acid and lysophosphatidylcholine, did not inhibit LPS-induced upregulation of E-selectin (Fig. 4a). Furthermore, only oxidized PAPC possessed the inhibitory activity, while non-oxidized PAPC or phosphatidylcholine containing saturated fatty acids (dimyristoylphosphatidylcholine, DMPC) were inactive (Fig. 4a and b). These data clearly show that oxidative modification is essential for phospholipids to gain anti-endotoxin activity. The inhibitory activity was not due to free-radical generation or free radicals already present in the OxPAPC preparations because addition of the antioxidant butylated hydroxytoluene had no effect (Fig. 4c). To identify individual lipid oxidation products responsible for the inhibitory activity, the lipid oxidation products contained in OxPAPC were fractionated using high-performance liquid chromatography/mass spectrometry (HPLC/MS)[9]. In fractions that inhibited LPS action, we consistently found a molecule with mass-to-charge ratio (m/z) = 594, previously identified as 1-palmitoyl-2-oxovalerolyl-*sn*-glycero-3-phosphorylcholine (POVPC)[9]. In fact, synthesized POVPC[8] inhibited LPS-induced E-selectin expression (Fig. 4d).

To determine whether OxPAPC exerts inhibitory effects also *in vivo*, we investigated LPS-induced inflammation at various levels of the inflammatory response, that is, NFκB activation, expression of inflammatory adhesion molecules and extravasation of inflammatory cells, using three established models of local and systemic LPS-induced inflammation in mice. In a skin model, OxPAPC inhibited LPS-induced accumulation of myeloperoxidase-expressing leukocytes at the site of injection (Fig. 3a, b). In an air pouch model[23], OxPAPC inhibited LPS-induced neutrophil immigration into the pouch cavity (Fig. 3c), p65 nuclear translocation in cells of the pouch wall (Fig. 3d) and VCAM-1 expression in endothelial cells as well as myofibroblasts in the pouch wall (Fig. 3e). After intraperitoneal injection of LPS, OxPAPC inhibited induction of E-selectin messenger RNA locally in the peritoneal wall, but also systemically in heart and aorta (Fig. 3f). Moreover, OxPAPC inhibited accumulation of blood-borne cells in the peritoneal cavity (Fig. 3g) and blocked oedema formation in the subserosal layer of the peritoneal wall (Fig. 3h). These data show that OxPAPC inhibits inflammatory effects of LPS also *in vivo*. Finally, OxPAPC significantly increased survival in mice injected with lethal doses of LPS (Fig. 3i).

Taken together, these results demonstrate that inhibition of LPS-induced acute inflammation by oxidized phospholipids suppresses innate immune responses both *in vitro* and *in vivo*. Our data clearly show that the anti-inflammatory action of OxPAPC is specific for LPS as compared to inflammatory cytokines TNFα and IL-1β. Thus, endogenously formed oxidized phospholipids potentially blunt the initiation of LPS-induced acute inflammation, although previous data indicate that they propagate chronic inflammation[7]. Furthermore, OxPAPC inhibits action of endotoxin through a mechanism apparently different from simple LPS entrapment leading to the formation of biologically inactive complexes. Our data instead suggest that the inhibition of the LPS effects by OxPAPC is mediated by interference with LPS binding to LBP and CD14, thereby inhibiting LPS presentation to TLR4.

We also find that oxidation is crucial for PAPC to acquire the anti-endotoxin activity. Thus, oxidation renders phospholipids capable of interacting with components of the innate immune system and of modifying responses to Gram-negative bacterial products. Given that under normal conditions LPS antagonistic activity of PAPC is low, it may increase dramatically at sites of bacterial inflammation owing to high concentrations of neutrophil-derived reactive oxygen species inducing lipid peroxidation[5]. This raises the possibility that phospholipid oxidation serves as a negative feedback mechanism to downregulate acute inflammation in Gram-negative infection. In addition, we identify POVPC as one chemical structure exerting anti-endotoxin effects, a compound quite different from previously described LPS scavengers or LPS receptor antagonists such as polymyxin B or lipid-A like substances[15,16]. These findings open up the possibility of developing new anti-endotoxin drugs with distinct chemical and pharmacodynamic properties. □

Methods

Lipids and lipopolysaccharide

PAPC, DMPC, 1-palmitoyl-*sn*-glycero-3-phosphorylcholine (lysoPC), arachidonic acid and lipopolysaccharide from *E. coli* serotype 055:B5 were purchased from Sigma-Aldrich, Vienna, Austria. OxPAPC was obtained by air oxidation of dry PAPC as described previously[9] and was stored in chloroform at −70 °C. POVPC was synthesized as described[8]. Immediately before the experiment, lipids were dried in glass tubes under the stream of N_2, and solubilized in culture medium by vigorous vortexing for 30 s. Where indicated, the lipids were additionally sonicated for 30 s.

Cells

Human umbilical-vein endothelial cells (HUVEC) were obtained and cultured as described[10]. Monolayers of HUVEC at passages 3 to 6 were treated with OxPAPC and agonists in medium 199 containing 10% supplemented calf serum or fetal calf serum (FCS) (HyClone). OxPAPC and other lipids were added to cells 20 min before the addition of stimuli. Longer preincubation did not produce stronger inhibition of the effects of LPS. In the absence of serum, the cells were insensitive to LPS.

Cell ELISA

For the enzyme-linked immunosorbent assay (ELISA), HUVEC were treated with lipids and agonists for 4 h, and then the cells were washed and fixed. Cell-surface-expressed E-selectin, ICAM-1 or VCAM-1 was detected using corresponding antibodies (R&D

Systems), secondary peroxidase-conjugated antibodies and *o*-phenylenediamine as substrate.

Quantification of NFκB-dependent transcription

Co-transfection of HUVEC with pNFκB-Luc (Stratagene) and pCMVβ (Clontech) was performed as described[10]. Two days after transfection, the cells were stimulated with 300 ng ml^{-1} LPS in the presence or absence of OxPAPC, and 6 h later were processed for luciferase and β-galactosidase activity measurements.

Analysis of p65/DNA binding

HUVEC were stimulated with 300 ng ml^{-1} LPS for 60 min in the presence or absence of 25 μg ml^{-1} OxPAPC. Analysis of p65 binding to its consensus oligonucleotide was performed in HUVEC lysates using the ELISA-based Trans-AM NFκB p65 kit (Active Motif).

Western blotting

HUVEC were stimulated with 300 ng ml^{-1} LPS or 20 U ml^{-1} TNFα in the presence or absence of 50 μg ml^{-1} OxPAPC. After 20 min (phospho-IκBα), 1 h (total IκBα) or 2 h (p38 MAPK), cells were scraped and analysed by western blotting as described[10].

Limulus amoebocyte lysate assay

OxPAPC aliquots (60 μg ml^{-1}) were mixed with equal volumes of LPS dilutions to produce final concentrations indicated on the *x*-axis in Fig. 2b. After 5 min at 37 °C, LAL reagent was added and LAL activity was determined by a quantitative chromogenic assay (Endochrome, Charles River Endosafe).

Binding of biotin–LPS to CD14 and LBP

LPS was biotinylated using EZ-Link Biotin-LC-Hydrazide (Pierce). Binding of biotin–LPS to recombinant human CD14 (Biometec) adsorbed onto ELISA plates was measured as described in ref. 24. Binding reaction was performed for 30 min at 37 °C in PBS/0.5% BSA buffer containing 1% FCS. In the absence of serum, binding of biotin–LPS to CD14 was below the detection limit. Bound biotin–LPS was detected with streptavidin–peroxidase and *o*-phenylenediamine. Binding of biotin–LPS to recombinant LBP (Biometec) captured by anti-LBP (HM14, a gift from W. A. Buurman) adsorbed to ELISA plates was measured as described in ref. 25. The binding was performed in PBS/0.1% BSA buffer. Detection of bound biotin-LPS was performed as described for CD14.

Animals

Experiments were performed according to Austrian animal rights law using female C57BL/6J mice (Institut für Labortierkunde und -genetik) of 20 g body weight.

Skin model of inflammation

LPS (10 μg) with or without OxPAPC (50 μg) in the final volume of 50 μl of 0.9% saline was injected intradermally into the abdominal region. After 24 h, mice were killed, and the skin at the injection site was excised and either embedded in optimal-cutting-temperature compound for immunohistochemistry or homogenized in potassium phosphate buffer (50 mM, pH 6.0) containing 0.5% hexadecyltrimethylammonium bromide. Sections of skin were stained for myeloperoxidase (MPO) using anti-MPO (from Dako) and Alexa 466-labelled secondary antibodies (Molecular Probes). Nuclei were stained with DAPI (Sigma-Aldrich). MPO activity in homogenates was monitored at 460 nm using H_2O_2 and *o*-dianisidine (Sigma-Aldrich) as substrates[26].

Air pouch model of inflammation

Air pouches of 5 ml were raised on day 0 on the dorsal surface of mice under light halothane anaesthesia, and a further 3 ml of air was injected 3 days later[23]. At day seven, 50 μg of LPS, 250 μg of OxPAPC, or a combination of both, was injected into the pouch in 1 ml of 0.9% saline. Twenty-four hours later, mice were killed, the pouches were lavaged and leukocytes were counted. Sections of air pouch wall were stained for p65 (Santa Cruz Biotechnology) or VCAM-1 (Southern Biotechnology Associates). Nuclei were stained with DAPI.

Peritoneal model of inflammation

Mice were injected intraperitoneally (i.p.) with 10 μg of LPS with or without 50 μg of OxPAPC at days 0 and 1. At day 2, the animals were killed and peritoneal leukocytes were counted in the lavage fluid. Paraffin-embedded sagittal sections of the anterior abdominal wall were stained with haematoxylin and eosin.

Survival studies

Mice were injected i.p. with 0.6 mg of LPS with or without 1.2 mg of OxPAPC in 0.5 ml of 0.9% saline. Survival was checked three times daily for up to 5 days.

mRNA expression

The mice were killed, and isolated organs were immersed into the RNAlater solution (Ambion). One μg of total RNA isolated using the RNAqueous 4PCR kit (Ambion) was reverse transcribed using GeneAmp RNA PCR core kit (Applied Biosystems).

Quantification of E-selectin mRNA was performed by real-time polymerase chain reaction (PCR) using the LightCycler instrument and Fast Start DNA Master SYBR Green I kit (Roche Diagnostics) as recommended by the manufacturer. The following primers were used: forward, 5′-GGTTTGGTGAGGTGTGCTC; reverse, 5′-TGATCTTTCCCGGAAC TGC. Each sample was also analysed for expression of β_2-microglobulin, which was used for normalization of E-selectin data.

Statistics

The data are presented as mean values ± standard deviations. The data are representative of at least two experiments producing similar results.

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1. Medzhitov, R. Toll-like receptors and innate immunity. *Nature Rev. Immunol.* **1**, 135–145 (2001).
2. Aderem, A. & Ulevitch, R. J. Toll-like receptors in the induction of the innate immune response. *Nature* **406**, 782–787 (2000).
3. Chow, J. C., Young, D. W., Golenbock, D. T., Christ, W. J. & Gusovsky, F. Toll-like receptor-4 mediates lipopolysaccharide-induced signal transduction. *J. Biol. Chem.* **274**, 10689–10692 (1999).
4. Beutler, B. Tlr4: central component of the sole mammalian LPS sensor. *Curr. Opin. Immunol.* **12**, 20–26 (2000).
5. Hampton, M. B., Kettle, A. J. & Winterbourn, C. C. Inside the neutrophil phagosome: oxidants, myeloperoxidase, and bacterial killing. *Blood* **92**, 3007–3017 (1998).
6. Zhang, R., Shen, Z., Nauseef, W. M. & Hazen, S. L. Defects in leukocyte-mediated initiation of lipid peroxidation in plasma as studied in myeloperoxidase-deficient subjects: systematic identification of multiple endogenous diffusible substrates for myeloperoxidase in plasma. *Blood* **99**, 1802–1810 (2002).
7. Lusis, A. J. Atherosclerosis. *Nature* **407**, 233–241 (2000).
8. Leitinger, N. *et al.* Structurally similar oxidized phospholipids differentially regulate endothelial binding of monocytes and neutrophils. *Proc. Natl Acad. Sci. USA* **96**, 12010–12015 (1999).
9. Watson, A. D. *et al.* Structurally identification by mass spectrometry of oxidized phospholipids in minimally oxidized low density lipoprotein that induce monocyte/endothelial interactions and evidence for their presence *in vivo*. *J. Biol. Chem.* **272**, 13597–13607 (1997).
10. Bochkov, V. N. *et al.* Oxidized phospholipids stimulate tissue factor expression in human endothelial cells via activation of ERK/EGR-1 and Ca^{++}/NFAT. *Blood* **99**, 199–206 (2002).
11. Elass-Rochard, E. *et al.* Lactoferrin inhibits the endotoxin interaction with CD14 by competition with the lipopolysaccharide-binding protein. *Infect. Immun.* **66**, 486–491 (1998).
12. Emancipator, K., Csako, G. & Elin, R. J. *In vitro* inactivation of bacterial endotoxin by human lipoproteins and apolipoproteins. *Infect. Immun.* **60**, 596–601 (1992).
13. Kitchens, R. L., Thompson, P. A., Viriyakosol, S., O'Keefe, G. E. & Munford, R. S. Plasma CD14 decreases monocyte responses to LPS by transferring cell-bound LPS to plasma lipoproteins. *J. Clin. Invest.* **108**, 485–493 (2001).
14. Wurfel, M. M. & Wright, S. D. Lipopolysaccharide-binding protein and soluble CD14 transfer lipopolysaccharide to phospholipid bilayers: preferential interaction with particular classes of lipid. *J. Immunol.* **158**, 3925–3934 (1997).
15. Rustici, A. *et al.* Molecular mapping and detoxification of the lipid A binding site by synthetic peptides. *Science* **259**, 361–365 (1993).
16. Christ, W. J. *et al.* E5531, a pure endotoxin antagonist of high potency. *Science* **268**, 80–83 (1995).
17. Golenbock, D. T., Hampton, R. Y., Qureshi, N., Takayama, K. & Raetz, C. R. Lipid A-like molecules that antagonize the effects of endotoxins on human monocytes. *J. Biol. Chem.* **266**, 19490–19498 (1991).
18. Tanamoto, K. & Azumi, S. Salmonella-type heptaacylated lipid A is inactive and acts as an antagonist of lipopolysaccharide action on human line cells. *J. Immunol.* **164**, 3149–3156 (2000).
19. Frey, E. A. *et al.* Soluble CD14 participates in the response of cells to lipopolysaccharide. *J. Exp. Med.* **176**, 1665–1671 (1992).
20. Su, G. L., Simmons, R. L. & Wang, S. C. Lipopolysaccharide binding protein participation in cellular activation by LPS. *Crit Rev. Immunol.* **15**, 201–214 (1995).
21. Sugiyama, T. & Wright, S. D. Soluble CD14 mediates efflux of phospholipids from cells. *J. Immunol.* **166**, 826–831 (2001).
22. Yu, B., Hailman, E. & Wright, S. D. Lipopolysaccharide binding protein and soluble CD14 catalyze exchange of phospholipids. *J. Clin. Invest.* **99**, 315–324 (1997).
23. Lawrence, T., Gilroy, D. W., Colville-Nash, P. R. & Willoughby, D. A. Possible new role for NF-κB in the resolution of inflammation. *Nature Med.* **7**, 1291–1297 (2001).
24. Cunningham, M. D. *et al. Helicobacter pylori* and *Porphyromonas gingivalis* lipopolysaccharides are poorly transferred to recombinant soluble CD14. *Infect. Immun.* **64**, 3601–3608 (1996).
25. Scott, M. G., Vreugdenhil, A. C., Buurman, W. A., Hancock, R. E. & Gold, M. R. Cutting edge: cationic antimicrobial peptides block the binding of lipopolysaccharide (LPS) to LPS binding protein. *J. Immunol.* **164**, 549–553 (2000).
26. Bradley, P. P., Priebat, D. A., Christensen, R. D. & Rothstein, G. Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. *J. Invest Dermatol.* **78**, 206–209 (1982).

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Competing interests statement

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